

09431594-110199

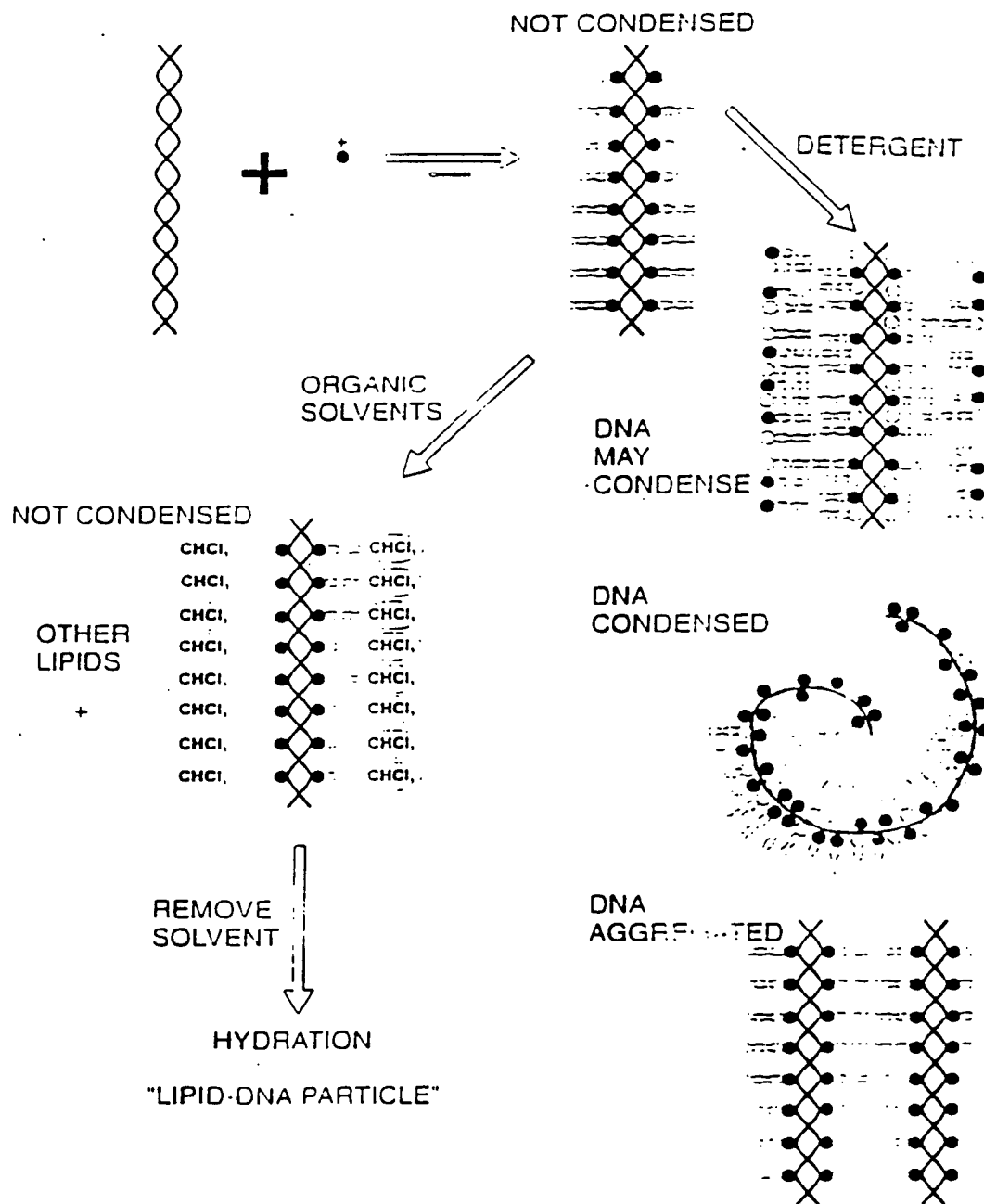


Figure 1

601451660

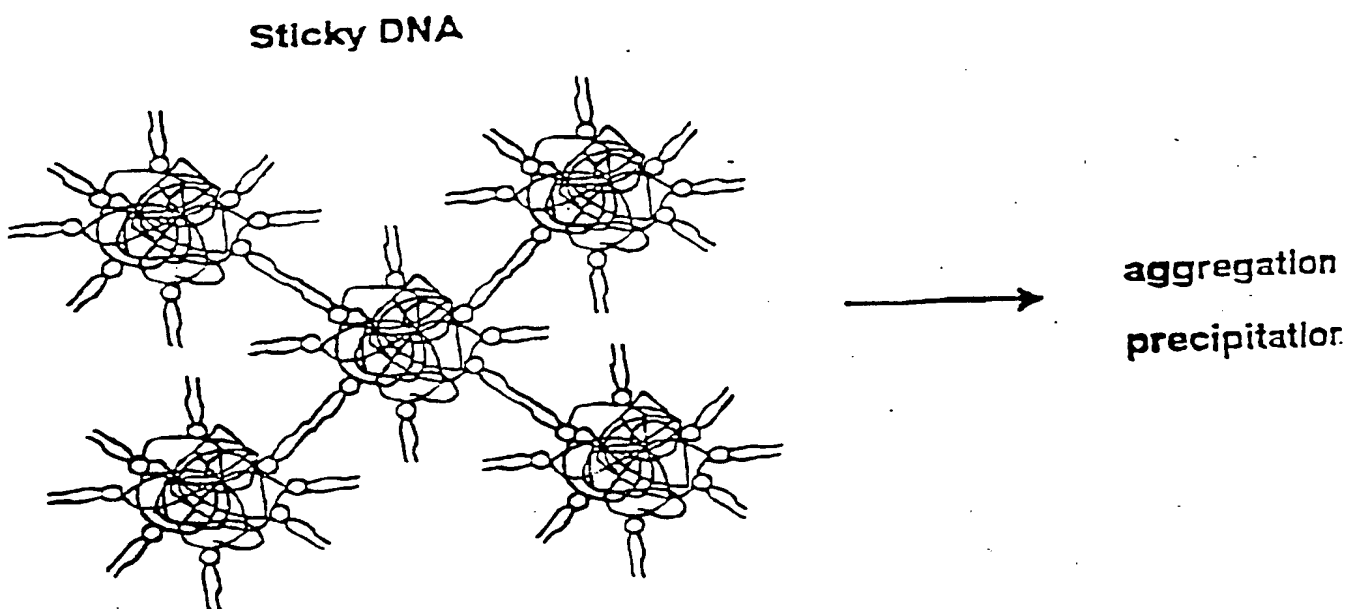
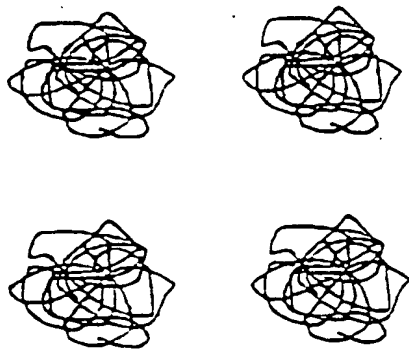


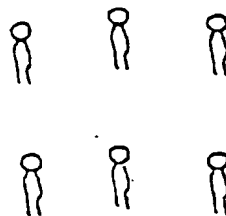
Figure 2

Negatively Charged Plasmid

Positively Charged Lipid

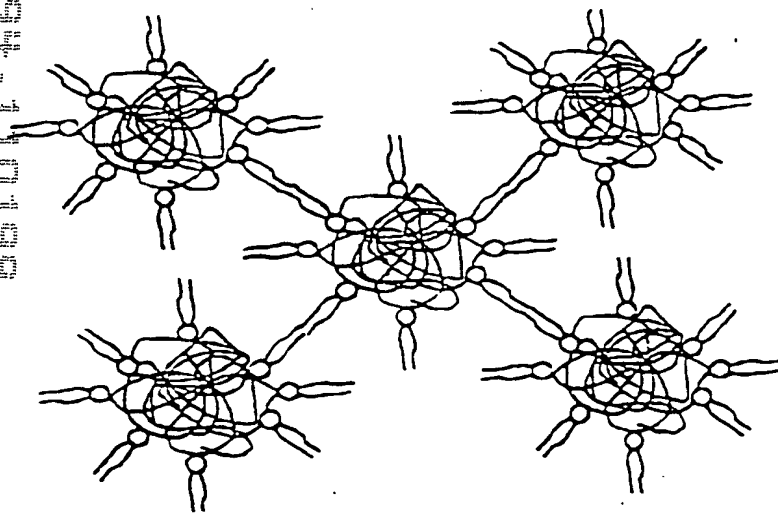


+

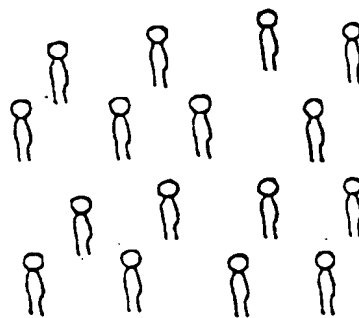


Sticky DNA

Excess Lipids



+



Plasmid

Encapsulated Within a Lipid Bilayer

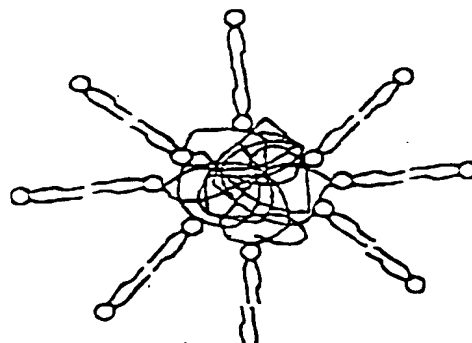
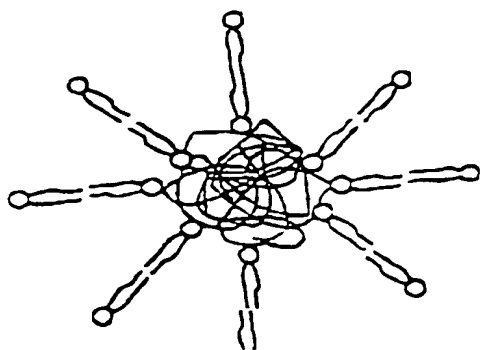


Figure 3

09431594 110199

Figure 1  
Recovery of DNA After Extrusion  
(20 mg total lipid)

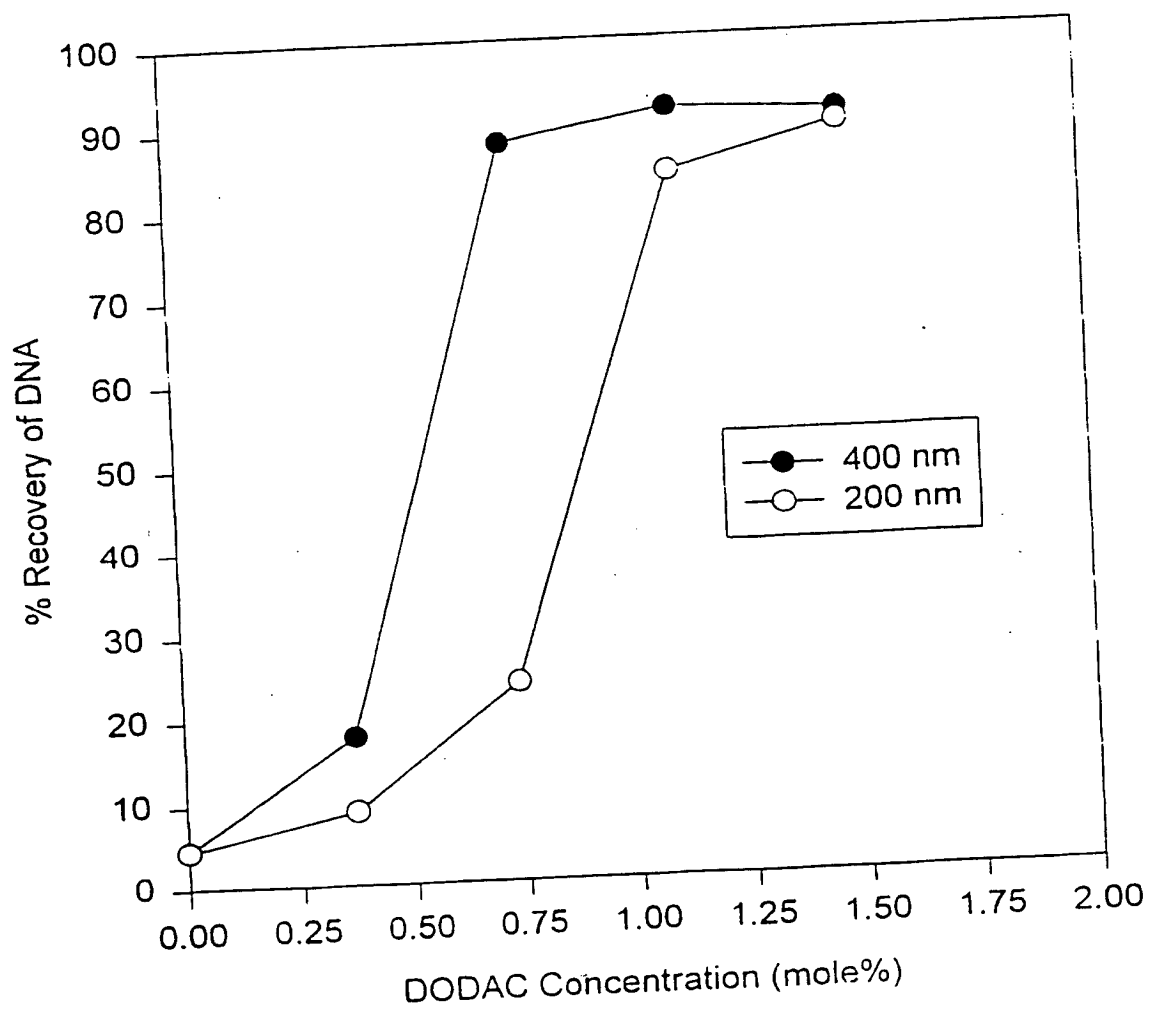


Figure 4

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INEX

026/043

Figure 2  
Anion Exchange Chromatography  
(20 mg total lipid)

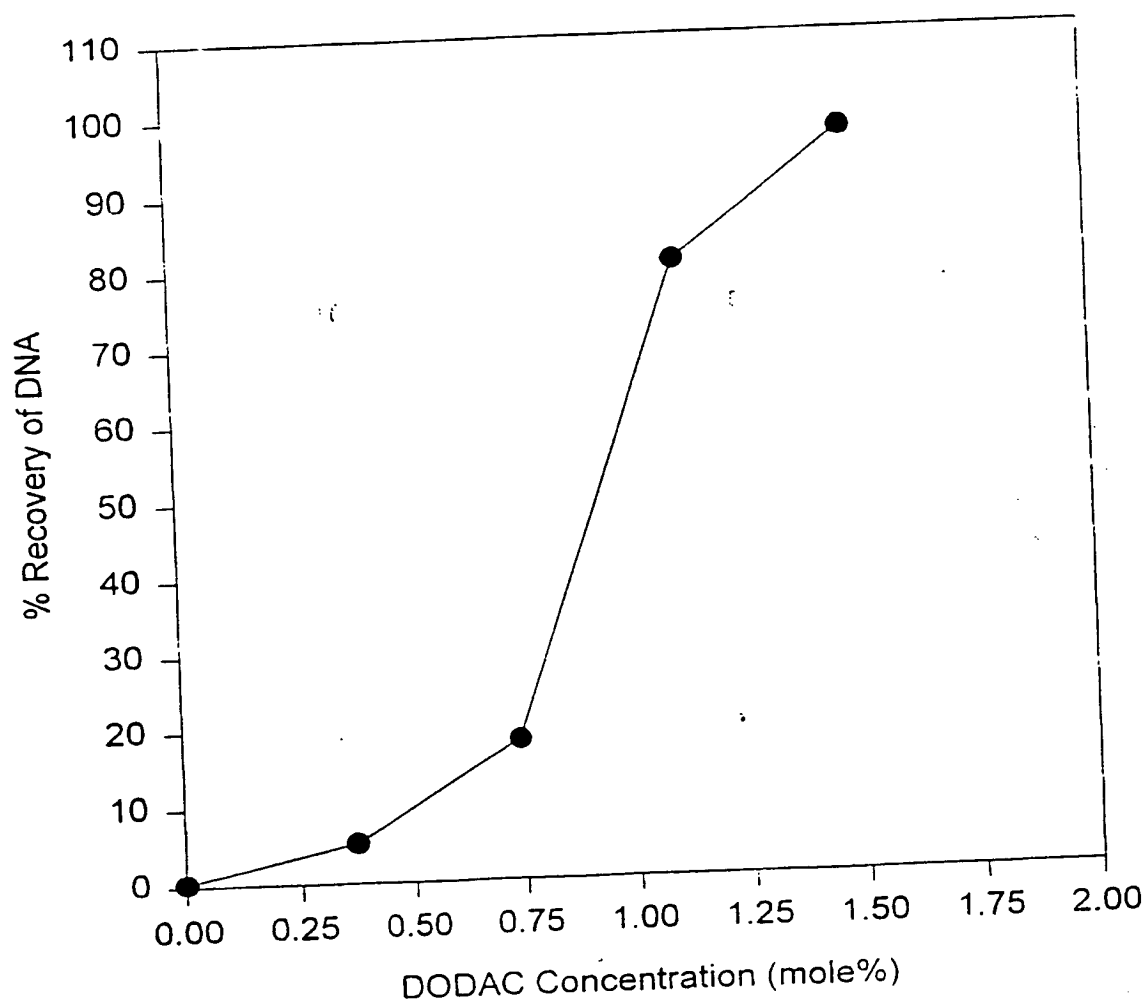


Figure 5

# Recovery of Lipid After Extrusion POPC:DODAC:PEG-Cer(C20), 20 mg

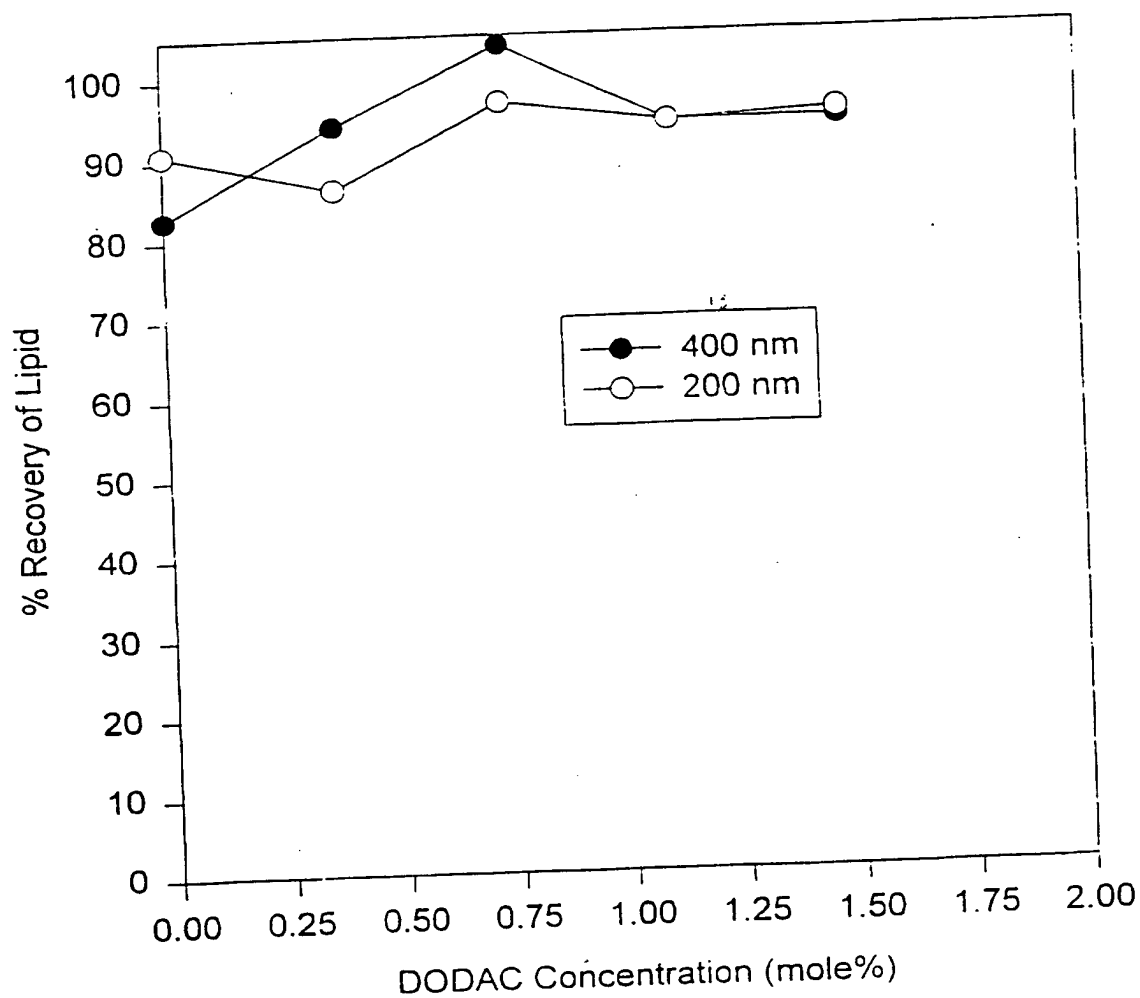


Figure 6

Anion Exchange Chromatography  
POPC:DODAC:PEG-Cer(C20), 20 mg

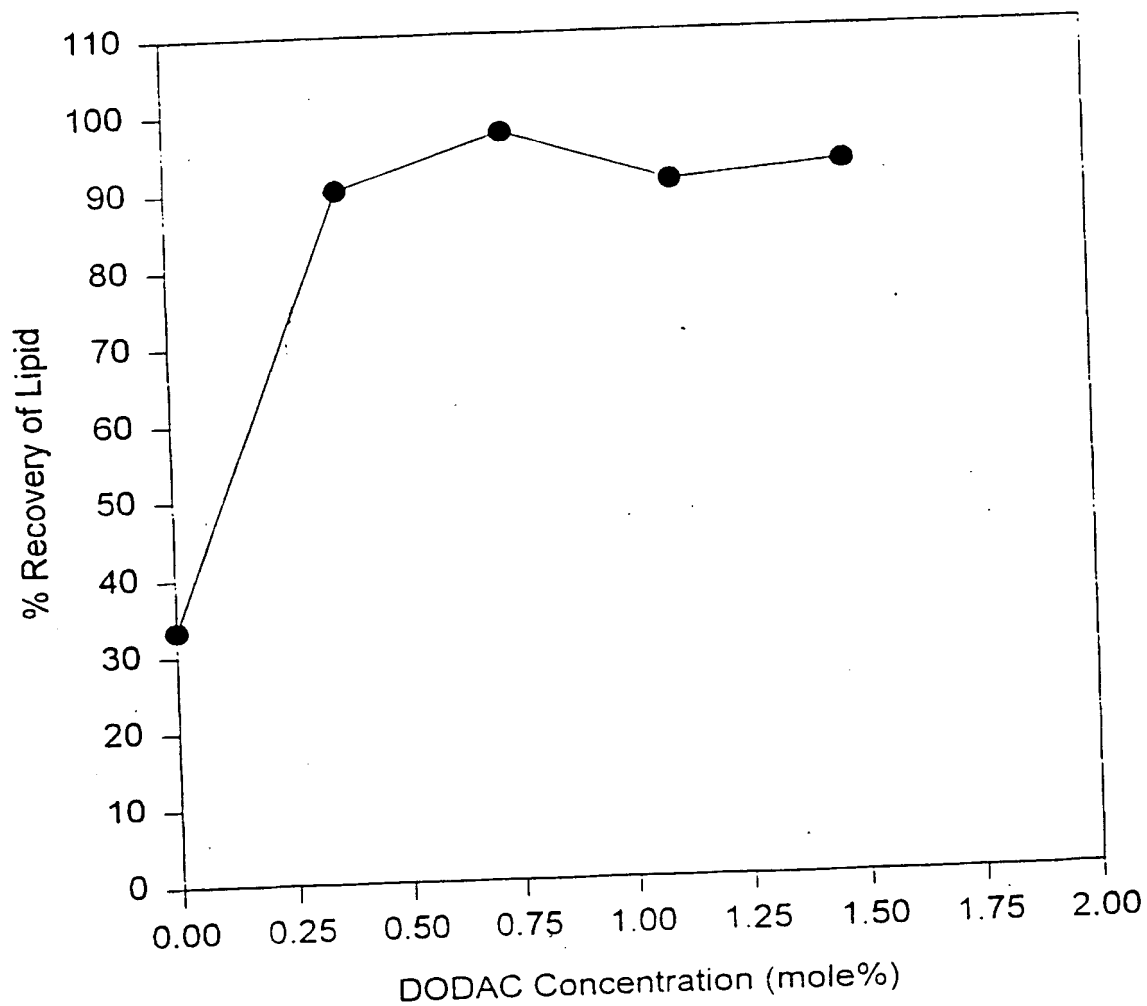


Figure 7

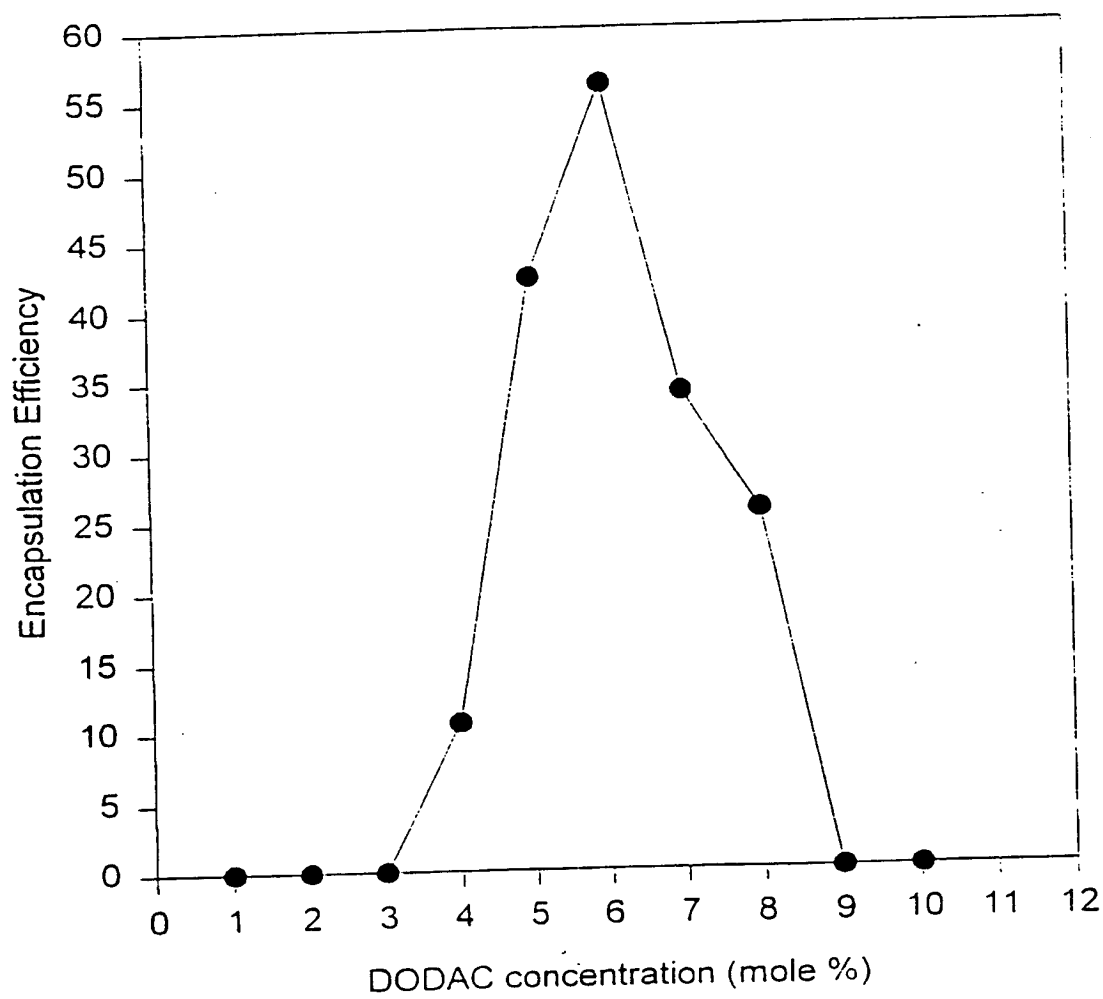


Figure 8



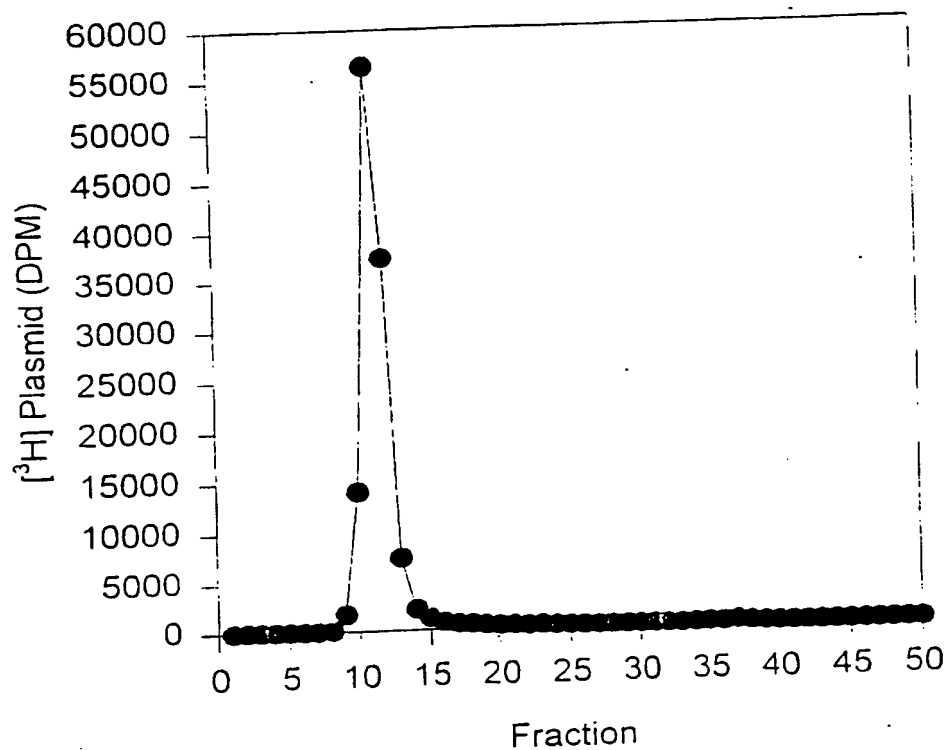


Figure 9A

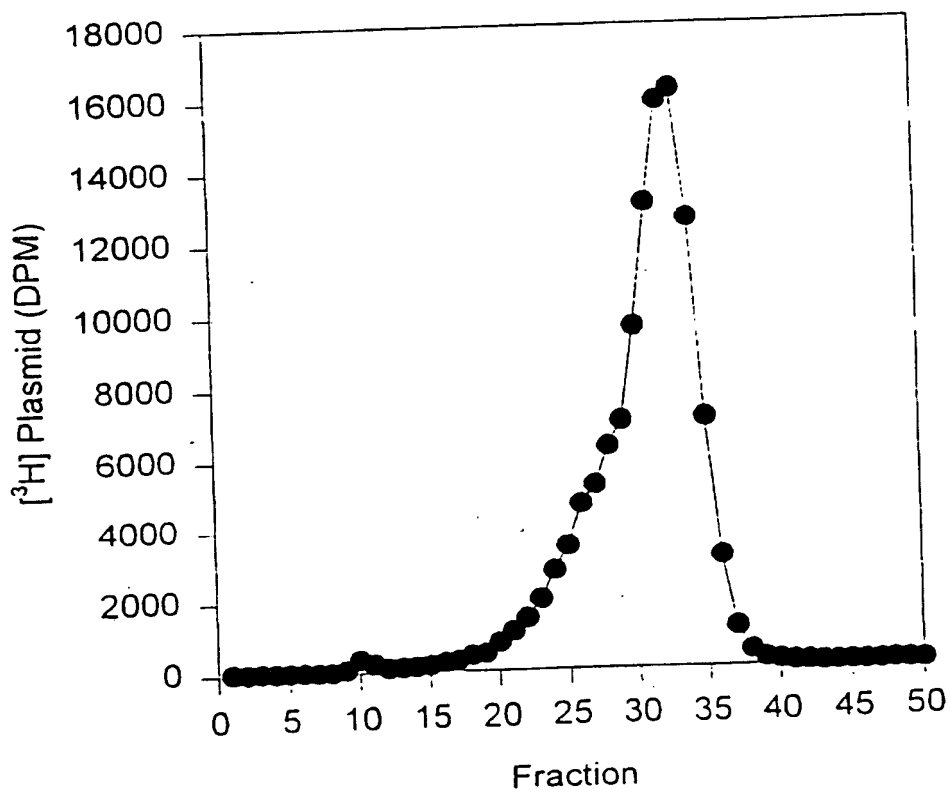


Figure 9B

05/31/96

02:35

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INEX

031/043

Recovery of  $^3\text{H}$  DNA and  $^{14}\text{C}$  Lipid  
After Incubation in Mouse Serum  
POPC:DODAC:PEG-Cer(C20)

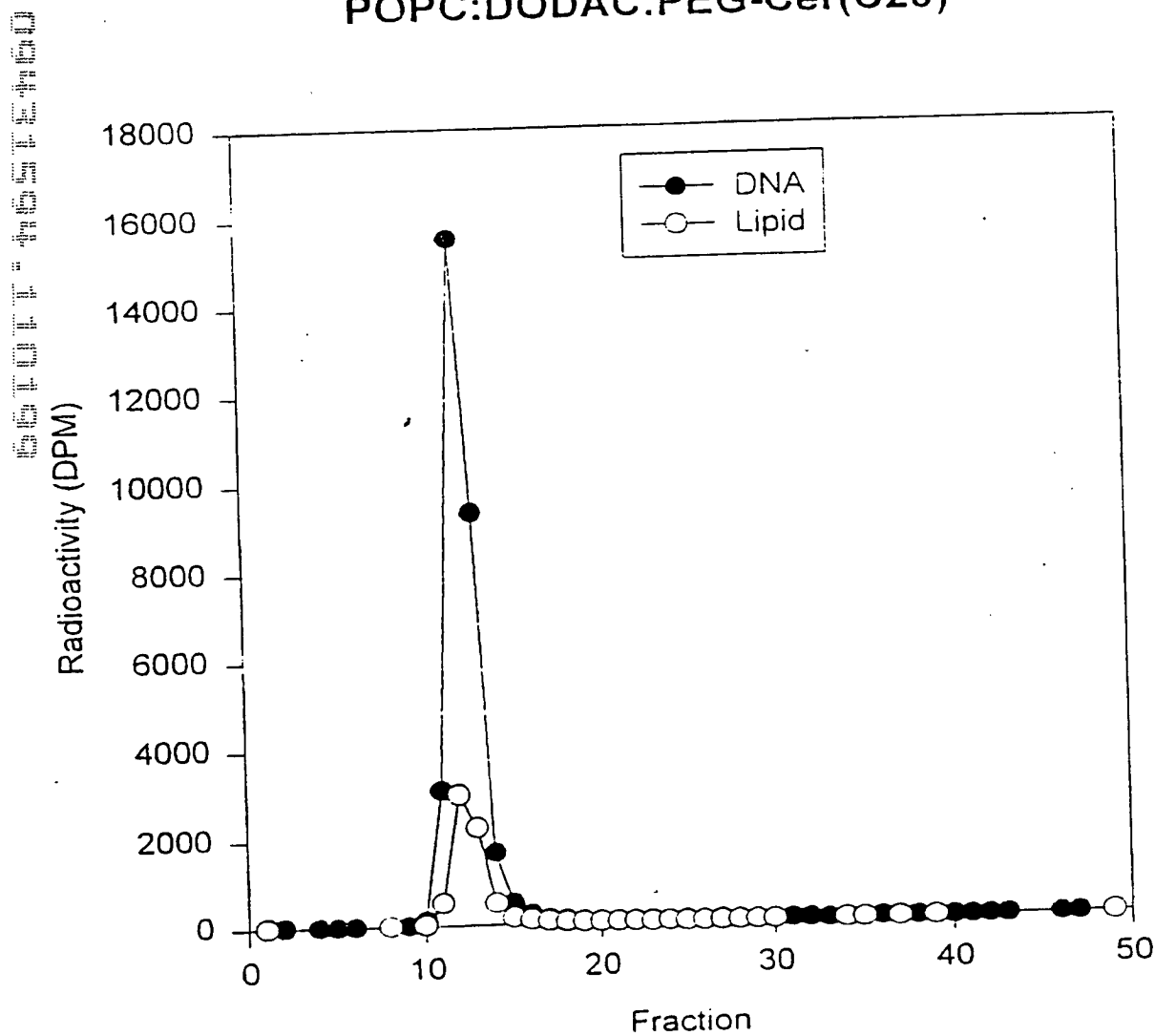


Figure 10

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02:35

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032/043

661016400

Figure 11A

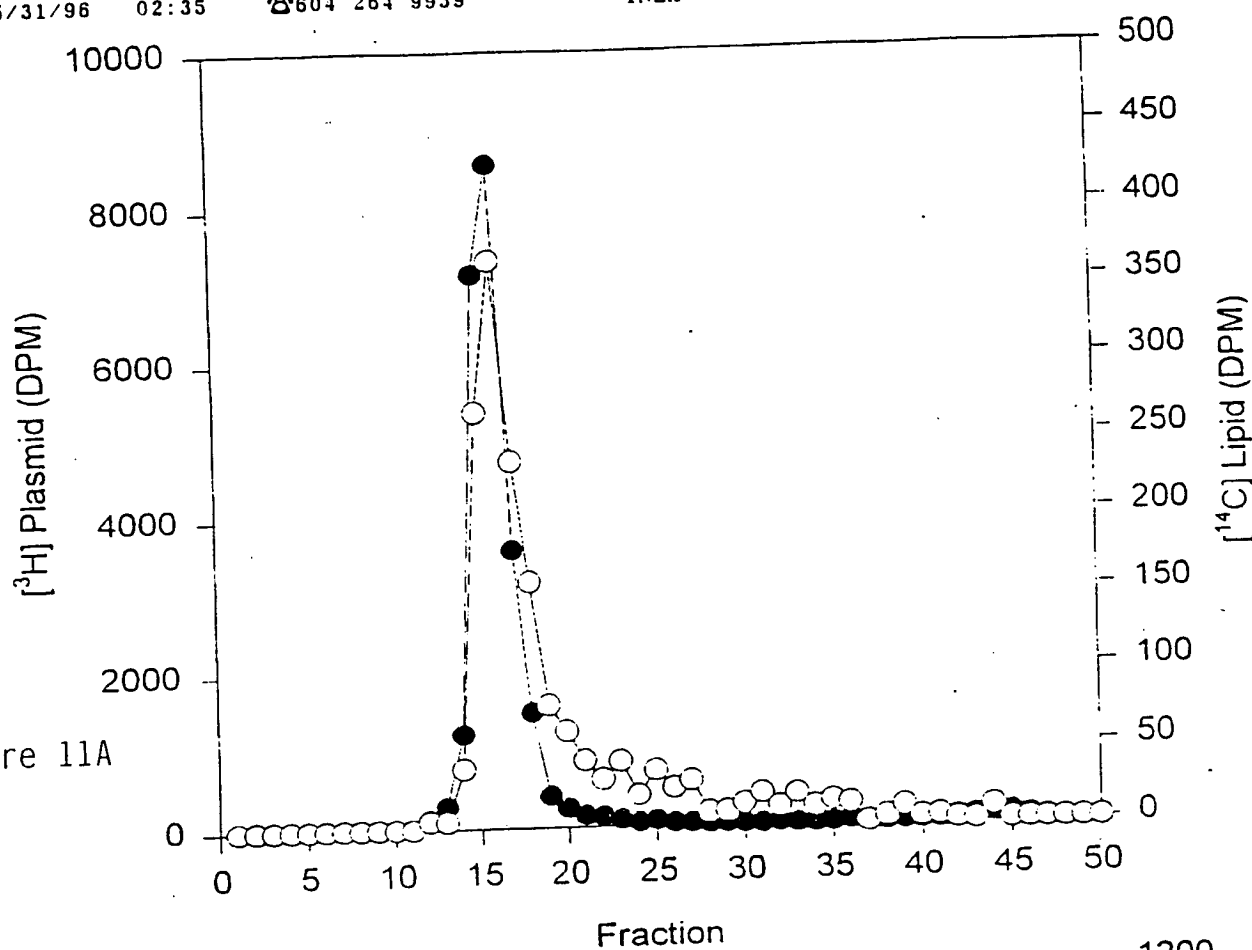
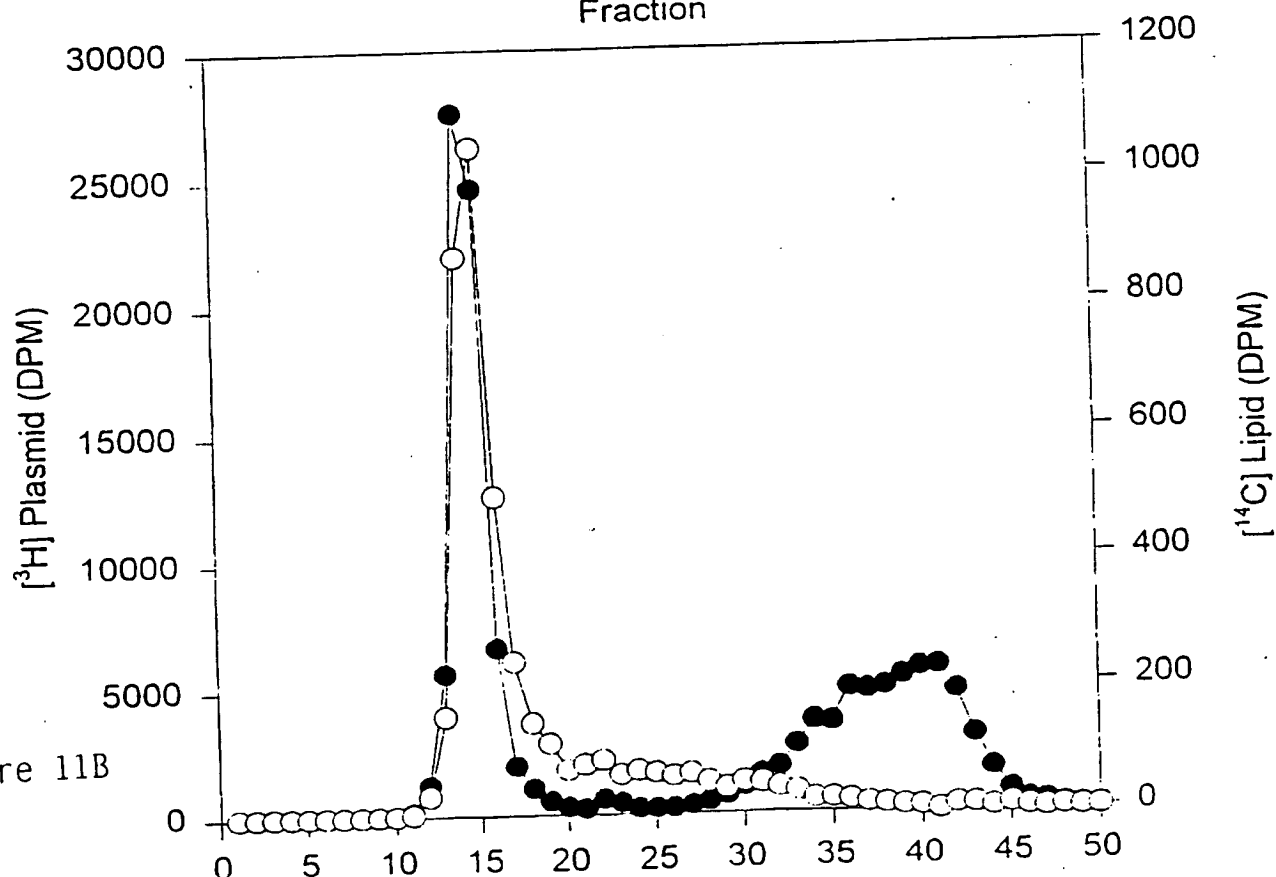


Figure 11B



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02:36

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INEX

033/043

A

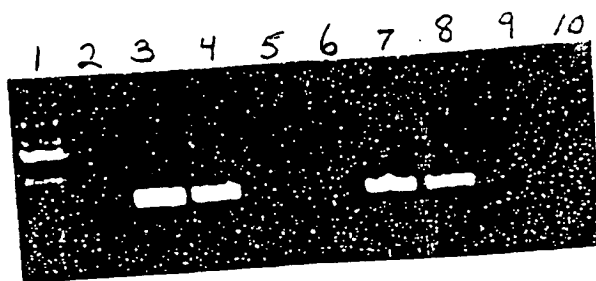


Figure 12A

B

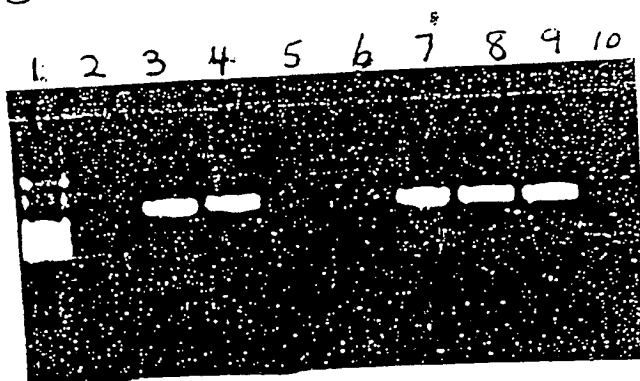


Figure 12B

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034/043

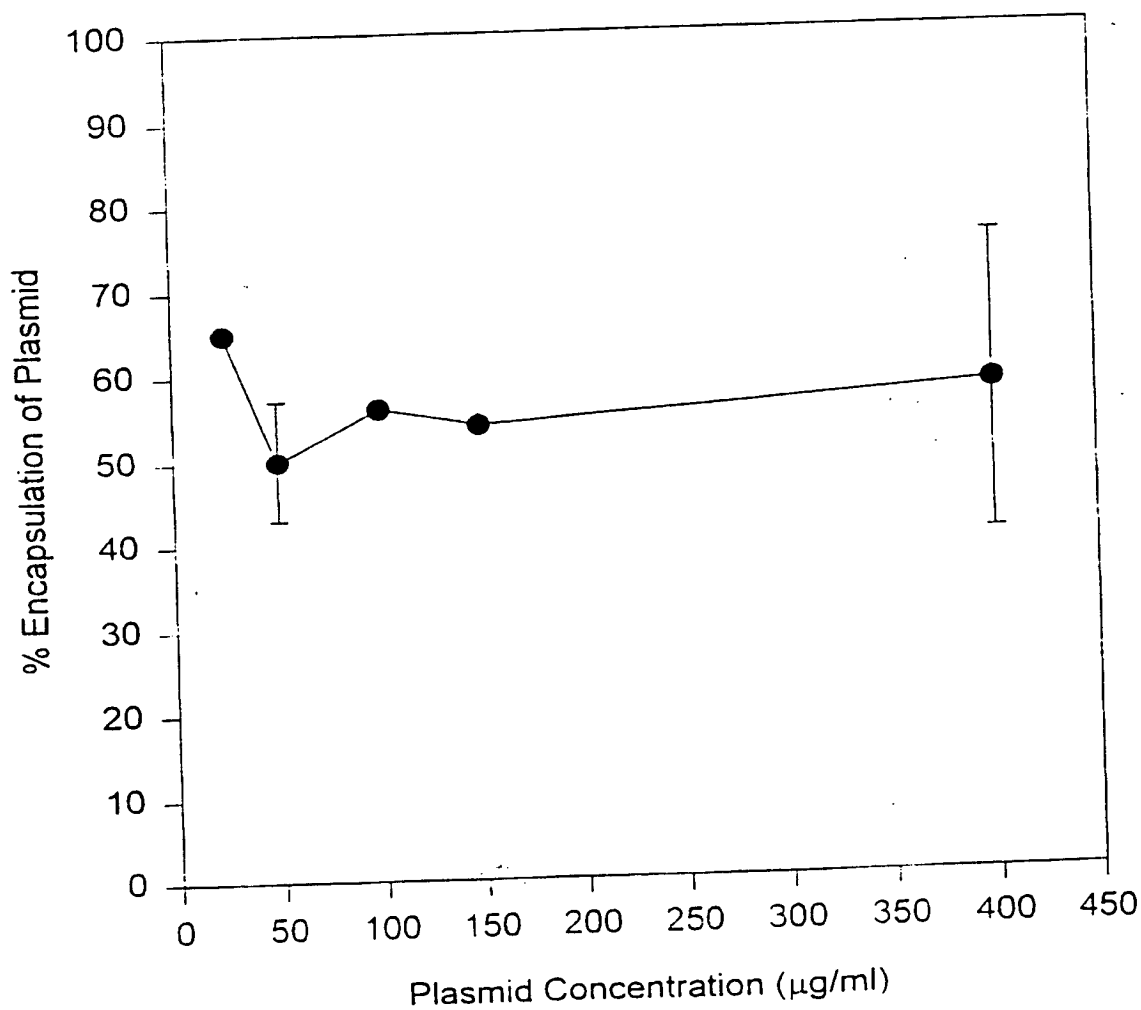


Figure 13

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INEX

☒ 035/043

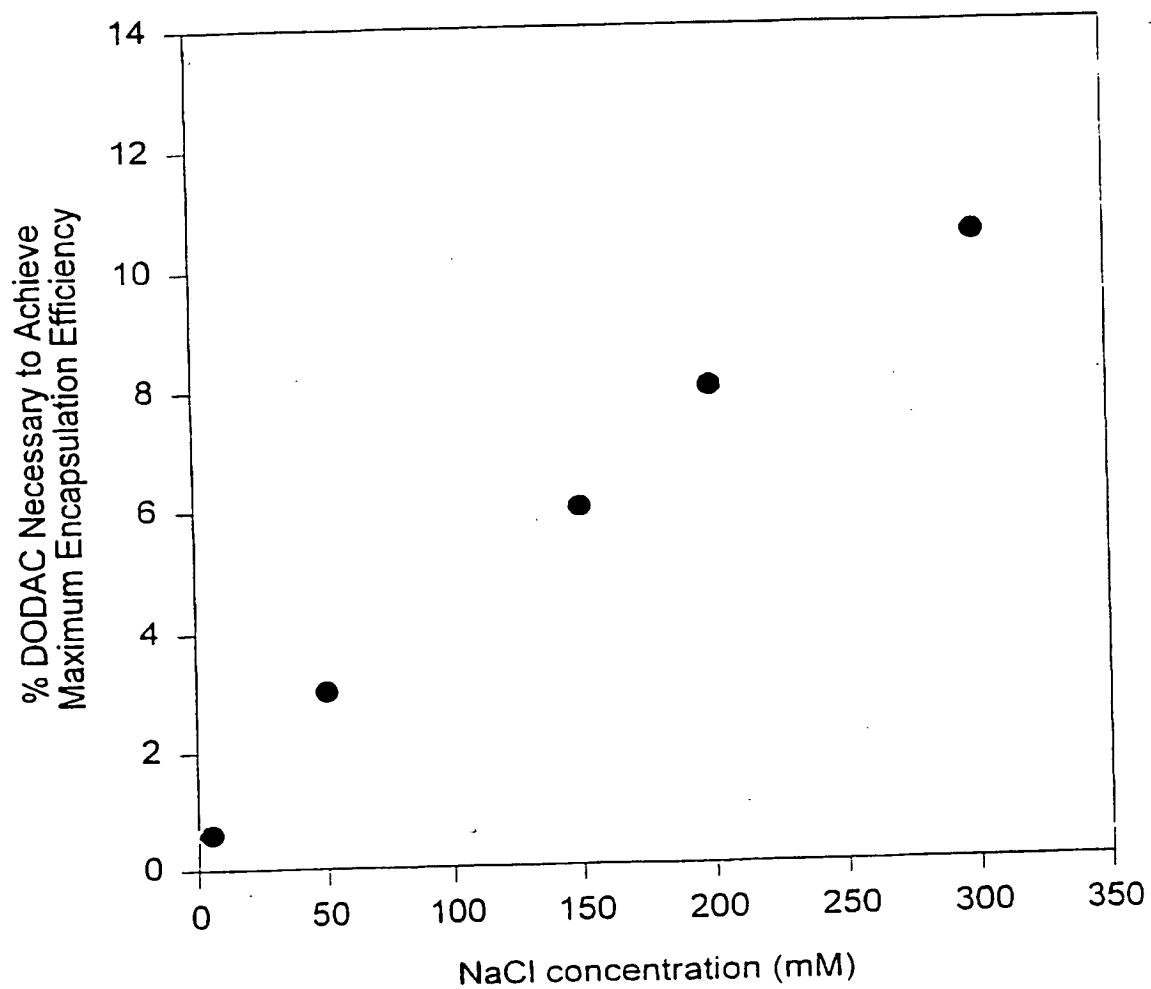


Figure 14

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INEX

036/043

DOPE:DODAC:PEG-Cer(C20) (84:6:10) for animal experiment  
Stored Data File a:\PEGC20.06

VOLUME-Weighted GAUSSIAN Analysis (Vesicles)

GAUSSIAN SUMMARY:

Mean Diameter = 61.6 nm

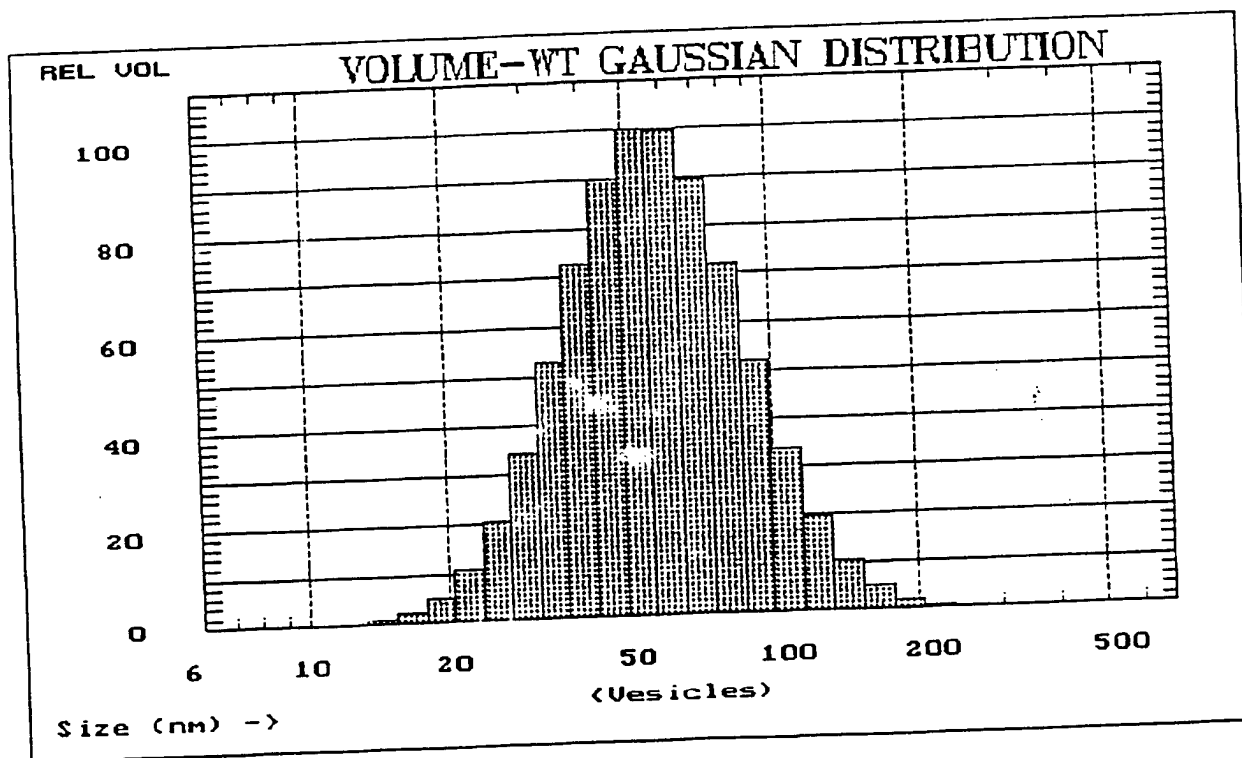
Std. Deviation = 27.0 nm (43.9 %)

Coeff. of Var'n = 0.439

Chi Squared = 0.347

Baseline Adj. = 0.000 %

Mean Diff. Coeff. = 7.54E-08 cm<sup>2</sup>/s



Cumulative Results:

25 % of distribution < 38.73 nm  
50 % of distribution < 52.05 nm  
75 % of distribution < 69.91 nm  
90 % of distribution < 91.36 nm  
99 % of distribution < 142.99 nm

Figure 15

Run Time = 1 Hr 43 Min 26 Sec  
Count Rate = 303 KHz  
Channel #1 = 2827.4 K  
Channel Width = 8.0 uSec

Wavelength = 632.8 nm  
Temperature = 23 deg C  
Viscosity = 0.933 cp  
Index of Ref. = 1.333

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INEX

037/043

DOPE:DODAC:PEG-Cer(C20) (84:6:10) for animal experiment  
Stored Data File a:\PEGC20.06

NUMBER-Weighted GAUSSIAN Analysis (Vesicles)

GAUSSIAN SUMMARY:

Mean Diameter = 32.8 nm

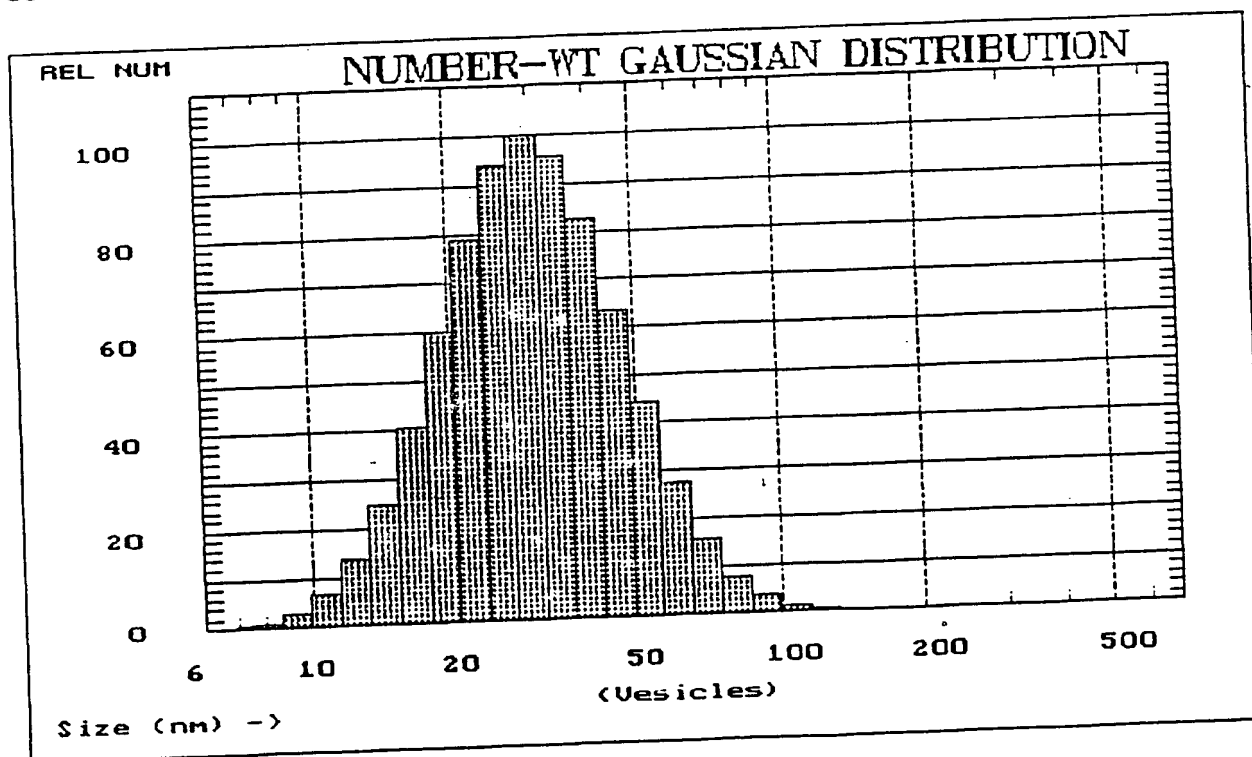
Std. Deviation = 14.4 nm (43.9 %)

Coeff. of Var'n = 0.439

Chi Squared = 0.347

Baseline Adj. = 0.000 %

Mean Diff. Coeff. = 1.42E-07 cm<sup>2</sup>/s



Cumulative Results:

25 % of distribution < 20.56 nm  
50 % of distribution < 27.72 nm  
75 % of distribution < 37.35 nm  
90 % of distribution < 48.88 nm  
99 % of distribution < 77.28 nm

Figure 16

Run Time = 1 Hr 43 Min 26 Sec  
Count Rate = 303 KHz  
Channel #1 = 2827.4 K  
Channel Width = 8.0 uSec

Wavelength = 632.8 nm  
Temperature = 23 deg C  
Viscosity = 0.933 cp  
Index of Ref. = 1.333



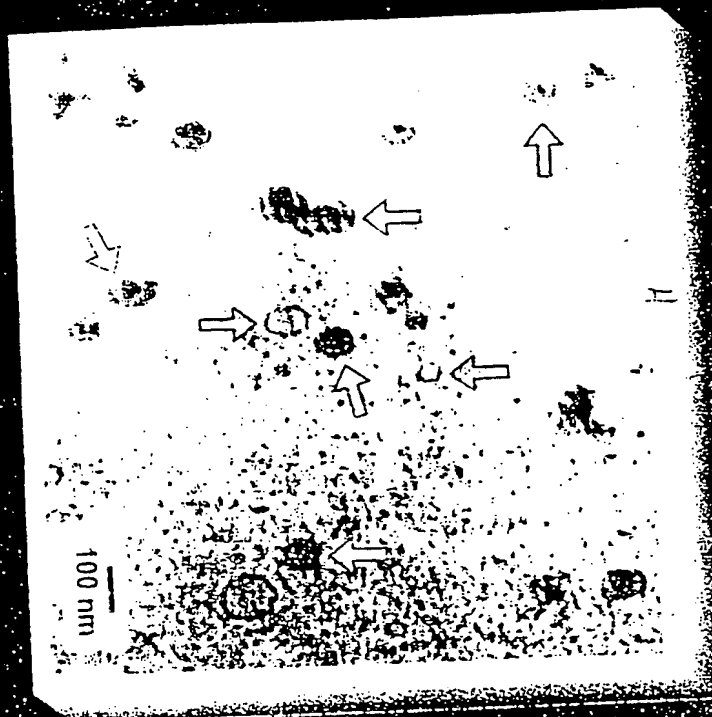


Figure 17A

# PLASMID TCS

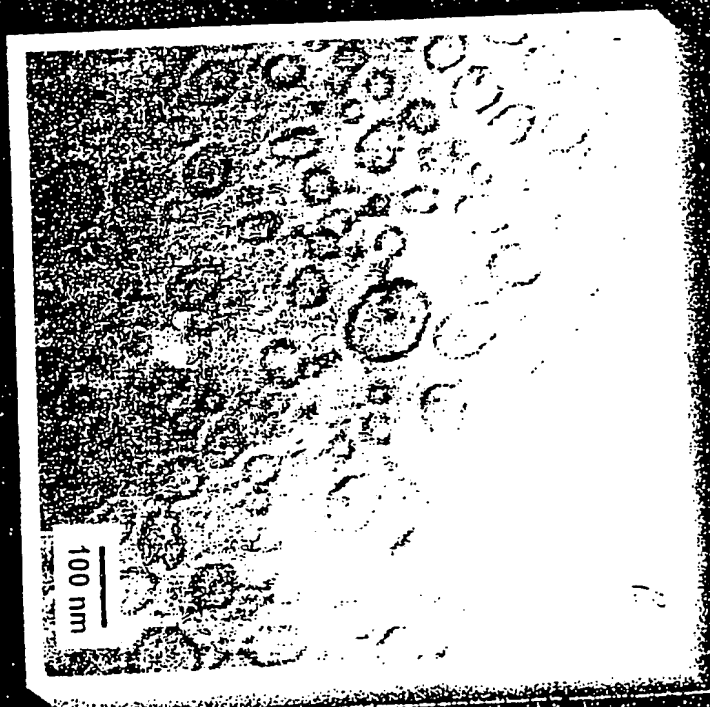


Figure 17B

### Clearance of DNA Encapsulated in POPC:DODAC:PEG-Cer(C20)

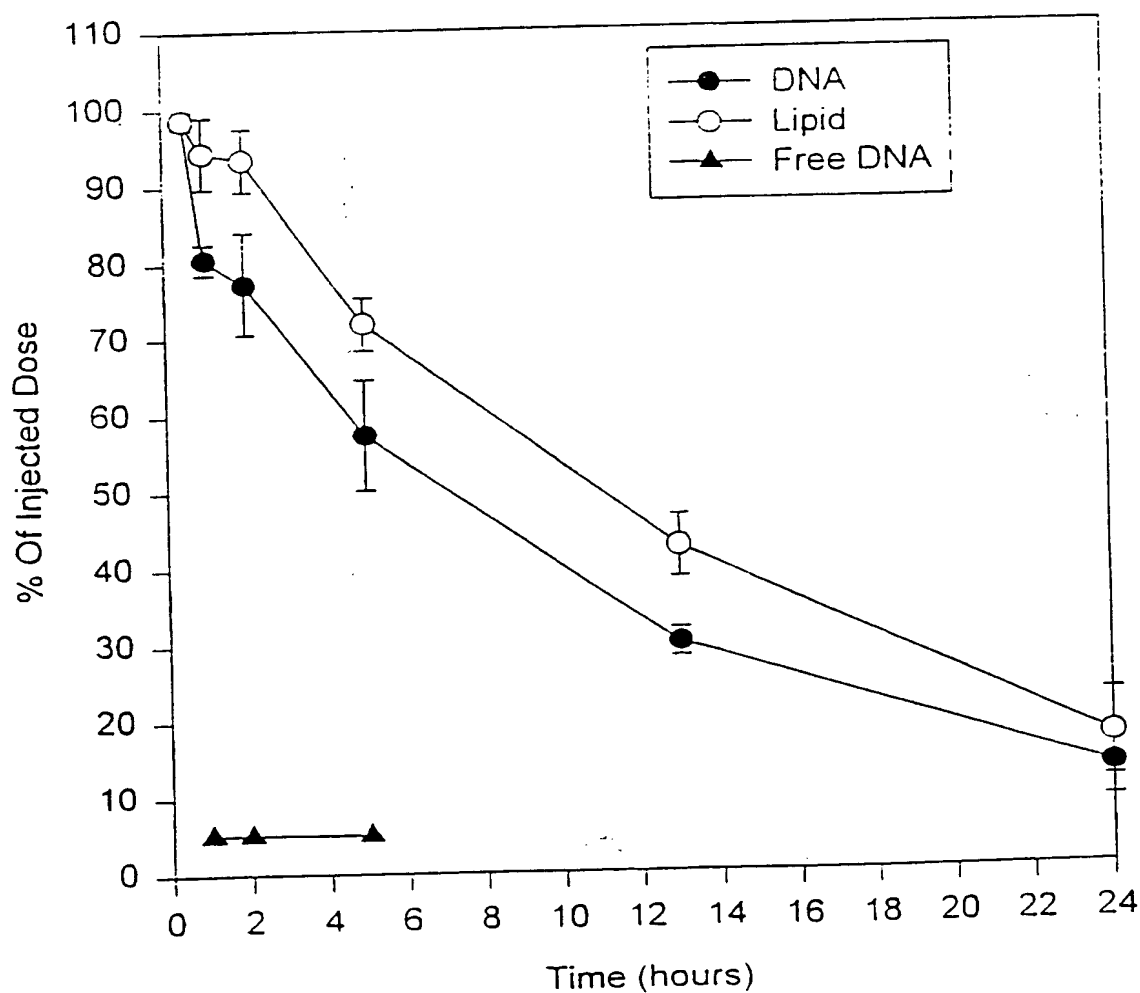


Figure 18

05/31/96

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604 264 9959

INEX

040/043

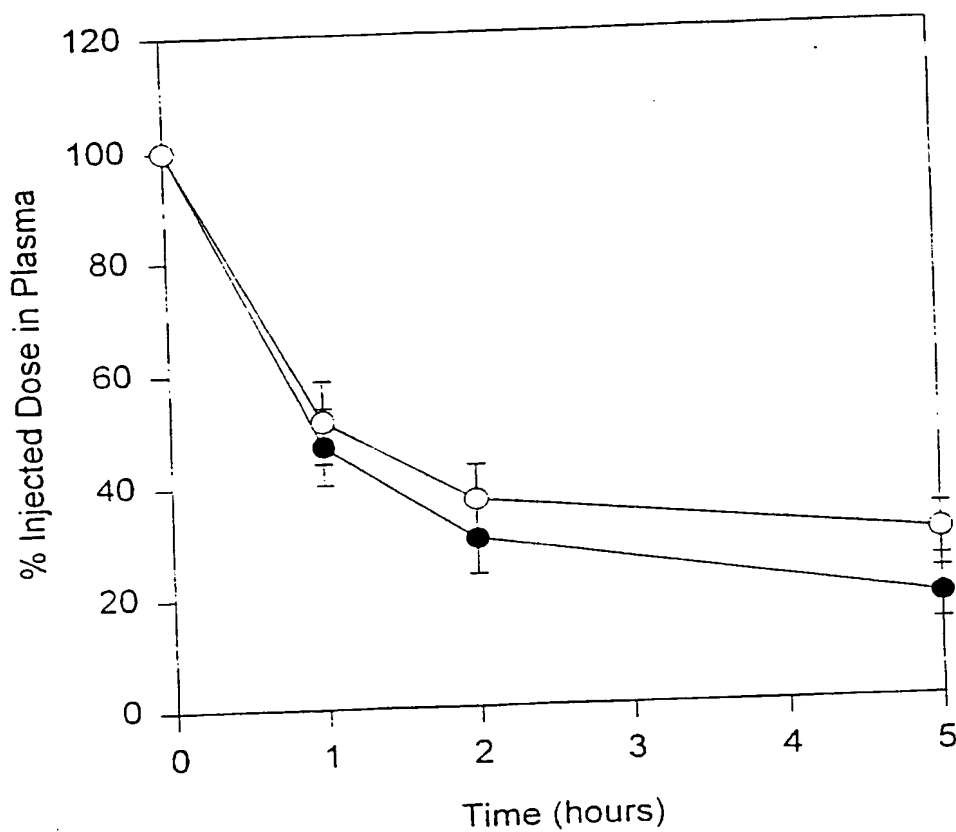


Figure 19A

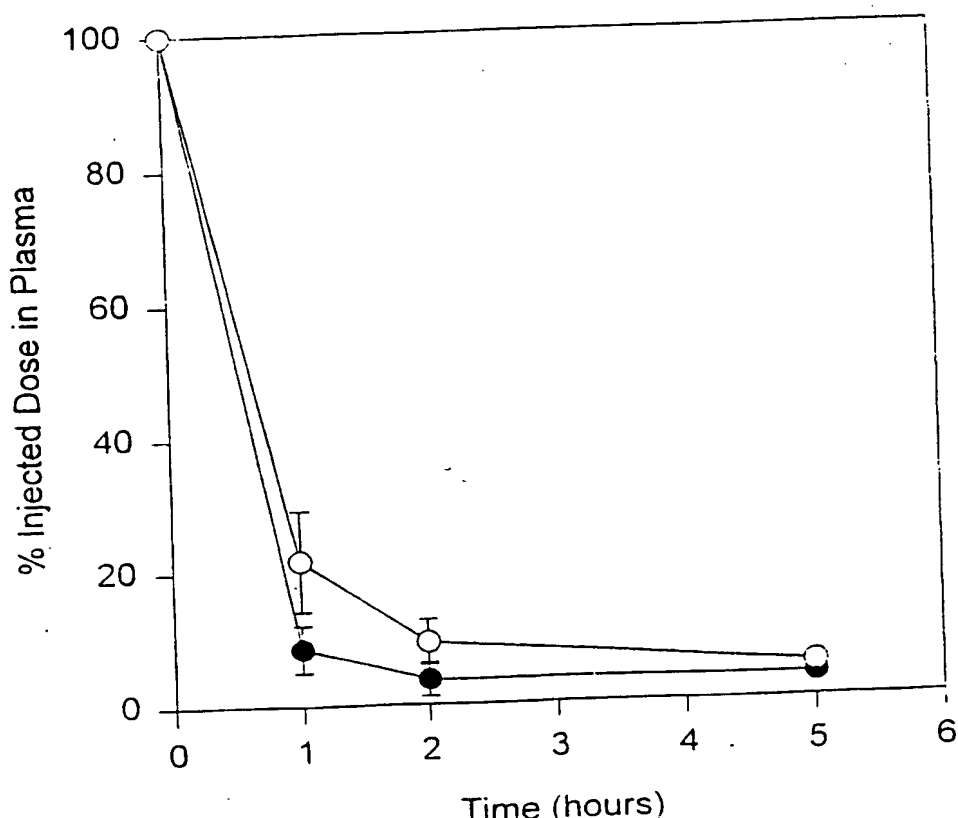


Figure 19B

### In Vivo Transfection in the Lung

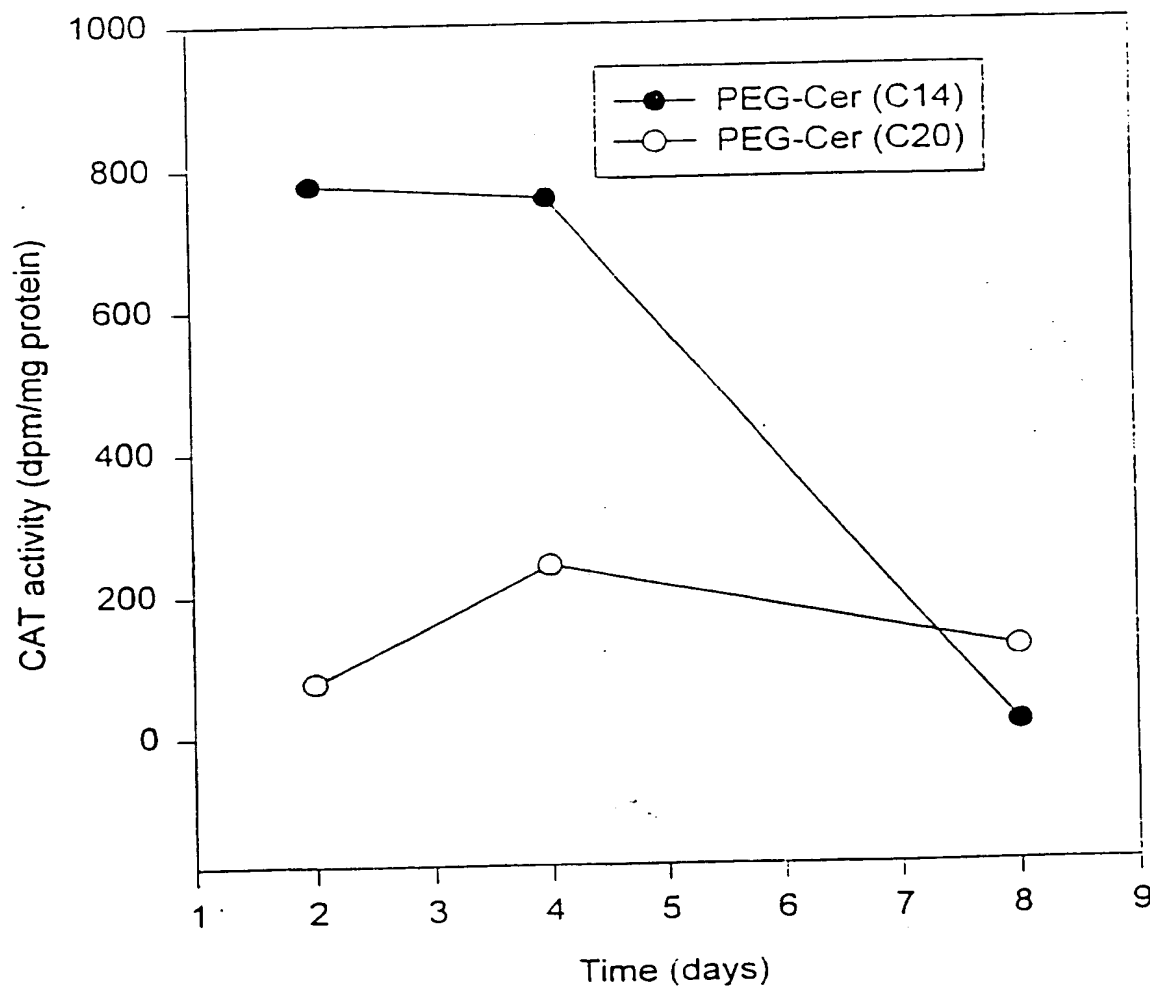


Figure 20

### In Vivo Transfection in the Liver

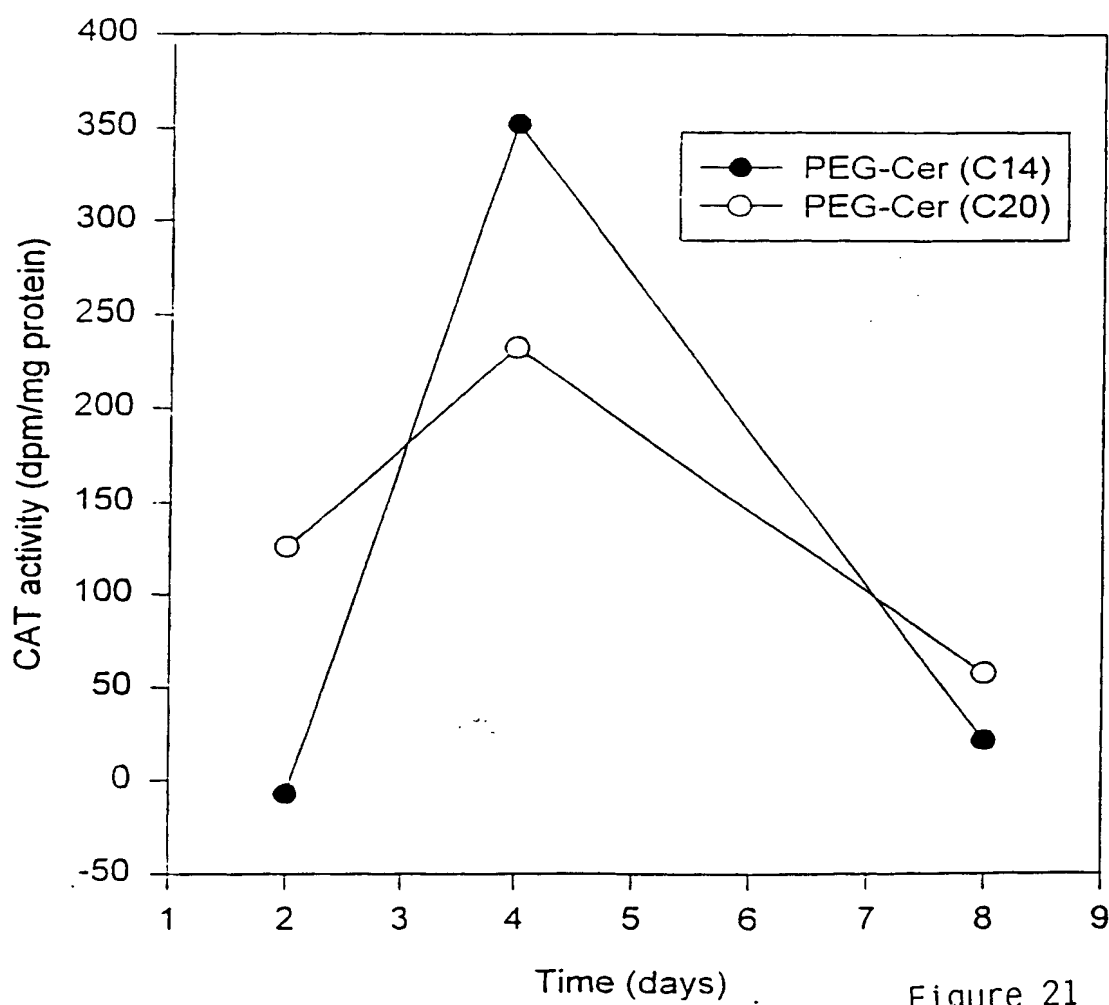


Figure 21

## In Vivo Transfection in the Spleen

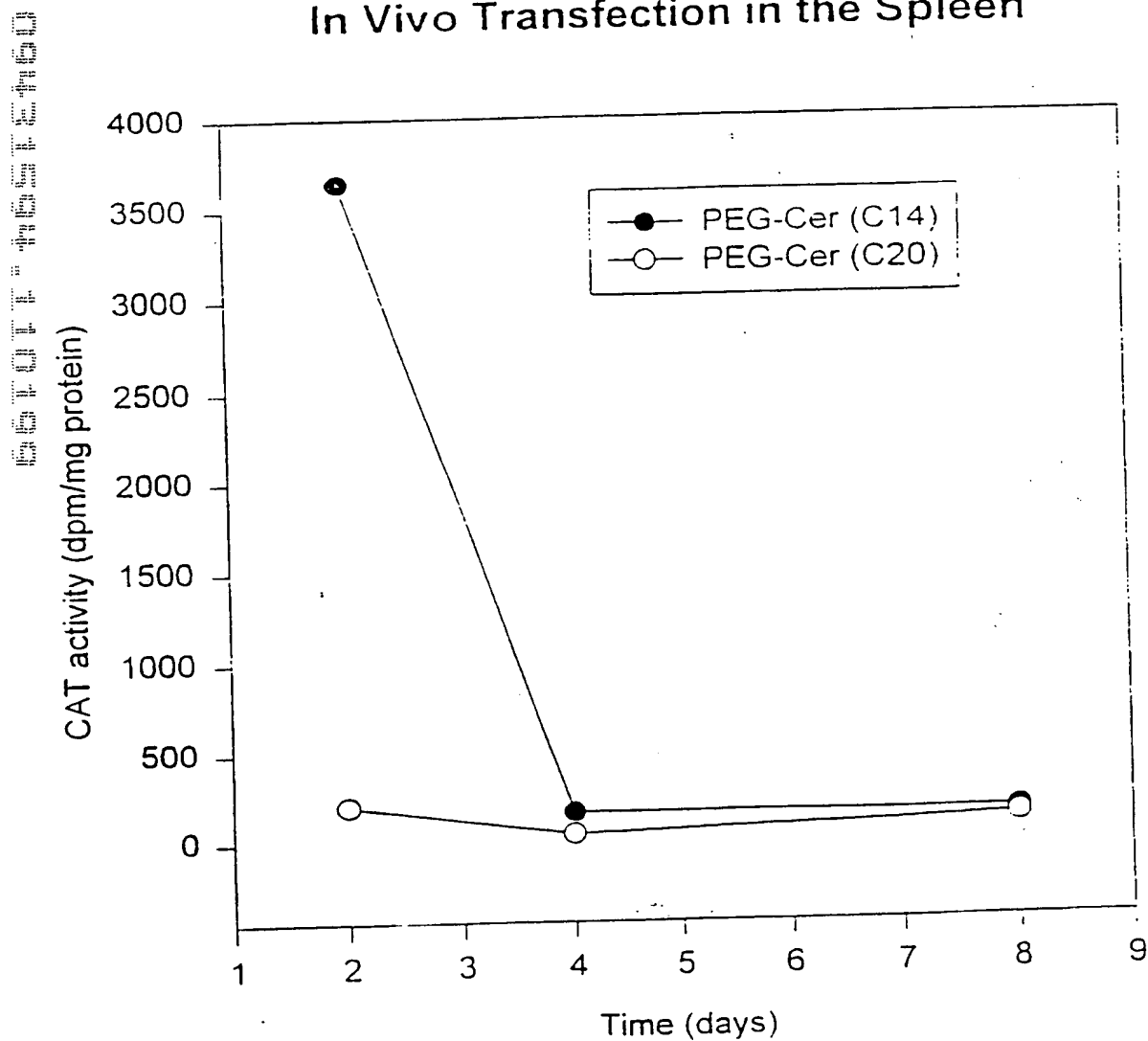


Figure 22

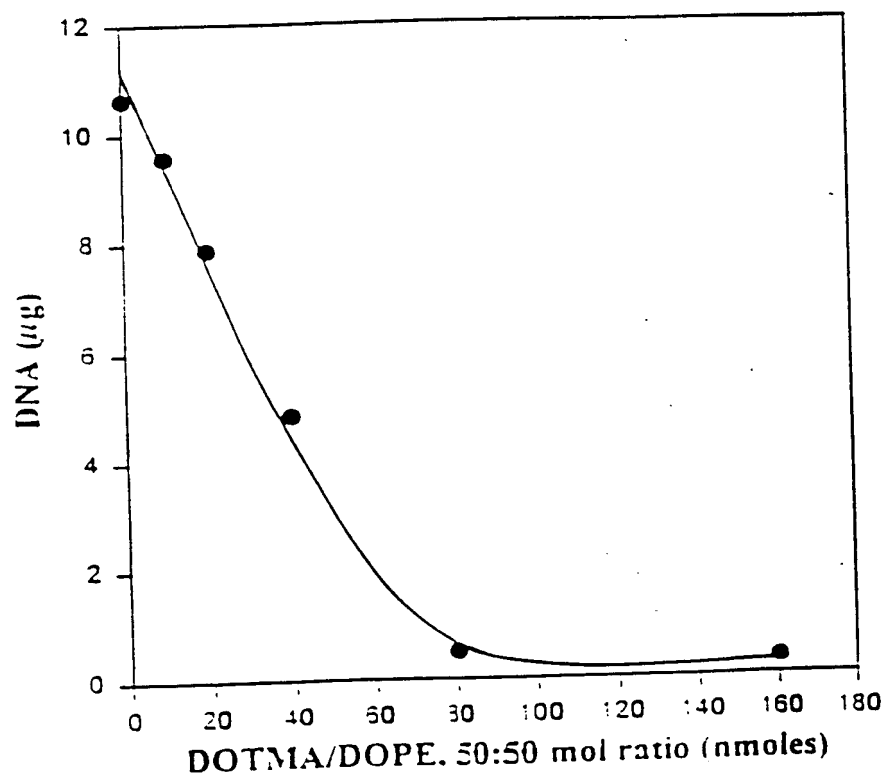


Figure 23

Figure 24A

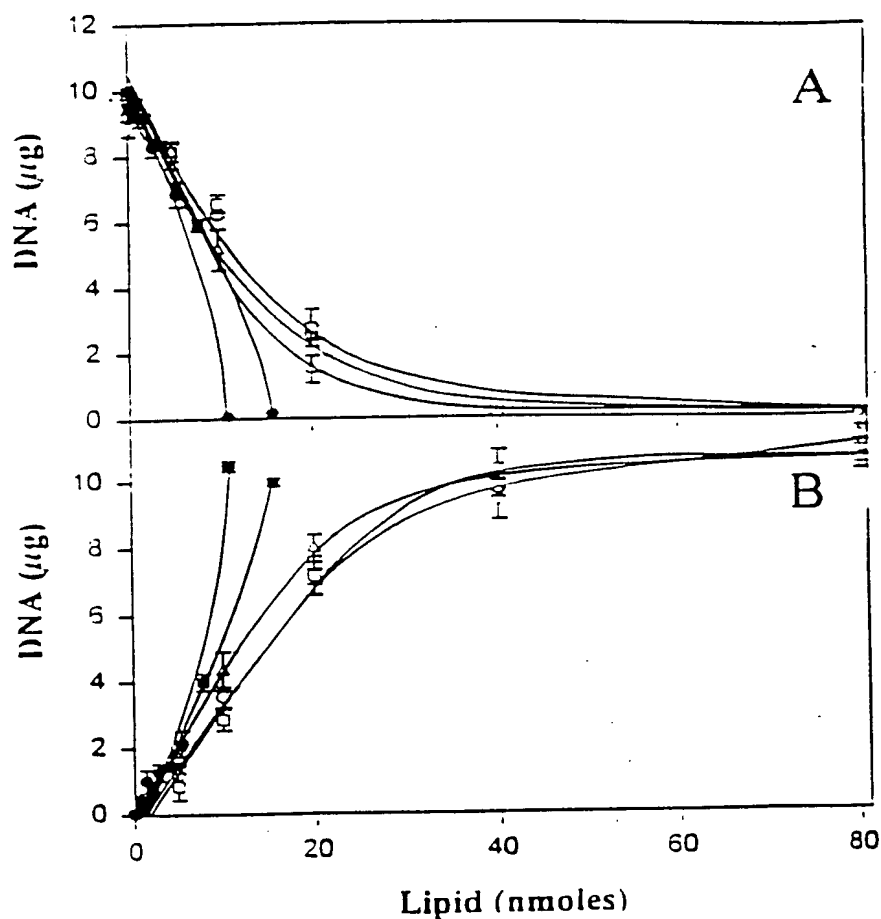


Figure 24B

Recovery of plasmid DNA in the aqueous (A) and solvent (B) phase following Bligh and Dyer extraction of the DNA/lipid complexes. DNA amount used was 10  $\mu\text{g}$ . Monocationic lipids used were DDAB (O), DOTMA ( $\square$ ) and DODAC ( $\Delta$ ). Lipopolyamines used were Lipofectamine ( $\bullet$ ), and Transfectam ( $\blacksquare$ ). All data points are averaged from three replications and expressed  $\pm$  SEM.



Figure 25A

Figure 25B

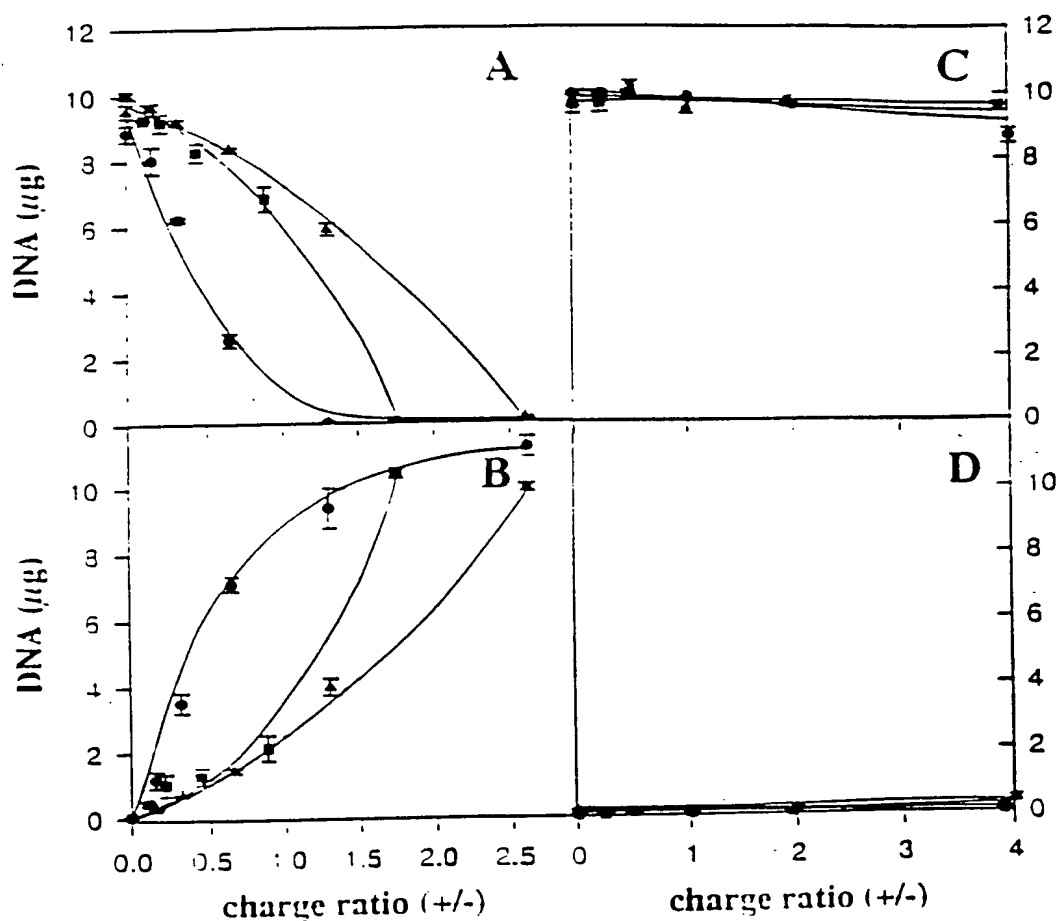


Figure 25C

Figure 25D

The recovery of plasmid DNA from aqueous (A and C) and solvent (B and D) fractions following Bligh and Dyer extractions and expressed as a function of charge ratio (+/-). (A and B), DDAB (●), Lipofectamine (■) and Transfectam (▲). (C and D), the effects of other cations, calcium (●), L-lysine (■), and poly-L-lysine (▲). DNA amount used was 10  $\mu$ g and all data points were averaged from three experiments and presented  $\pm$  SEM.

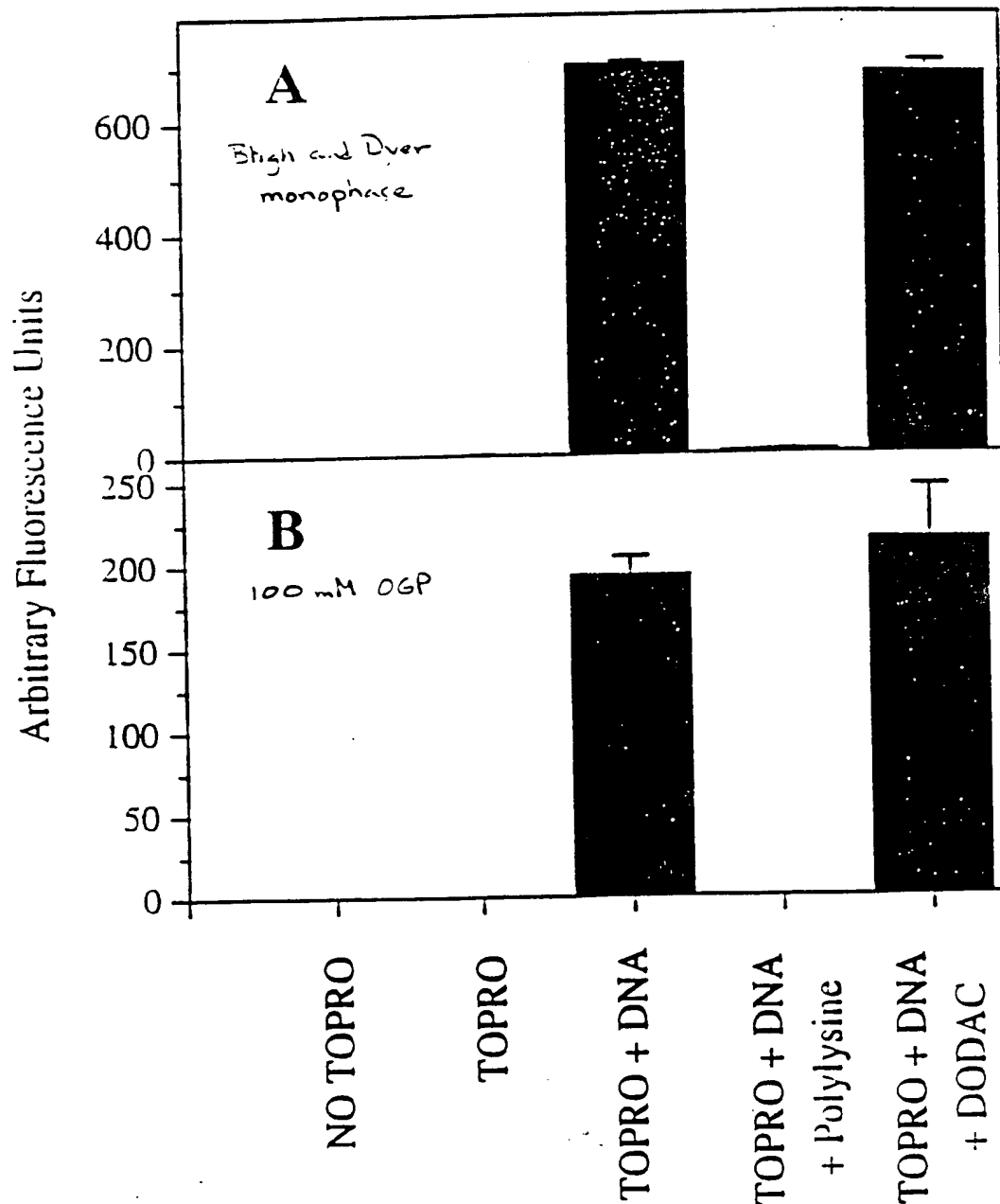
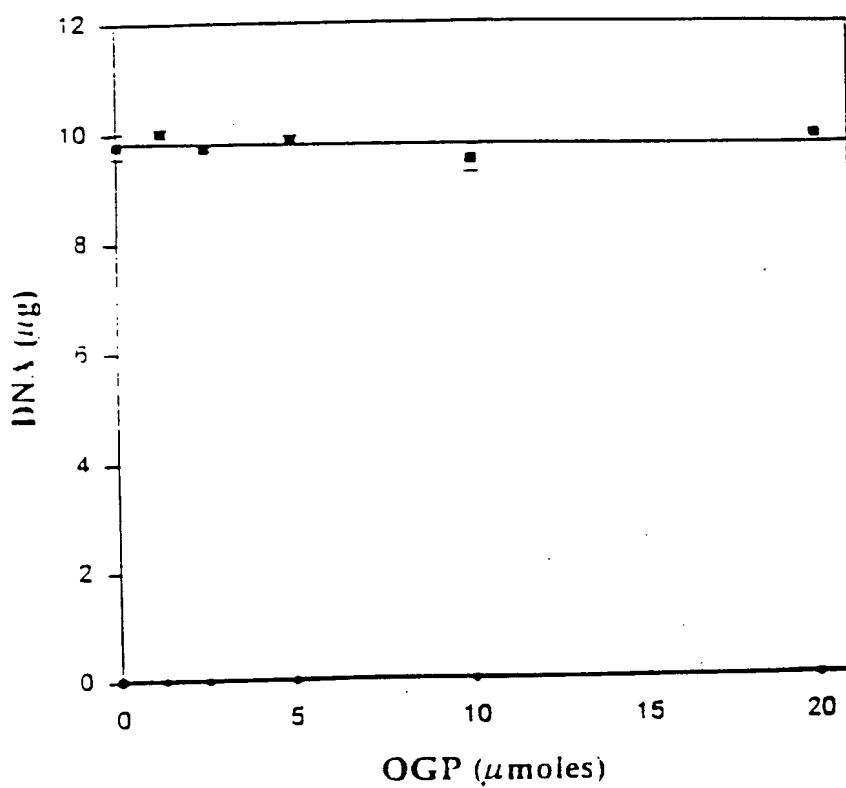


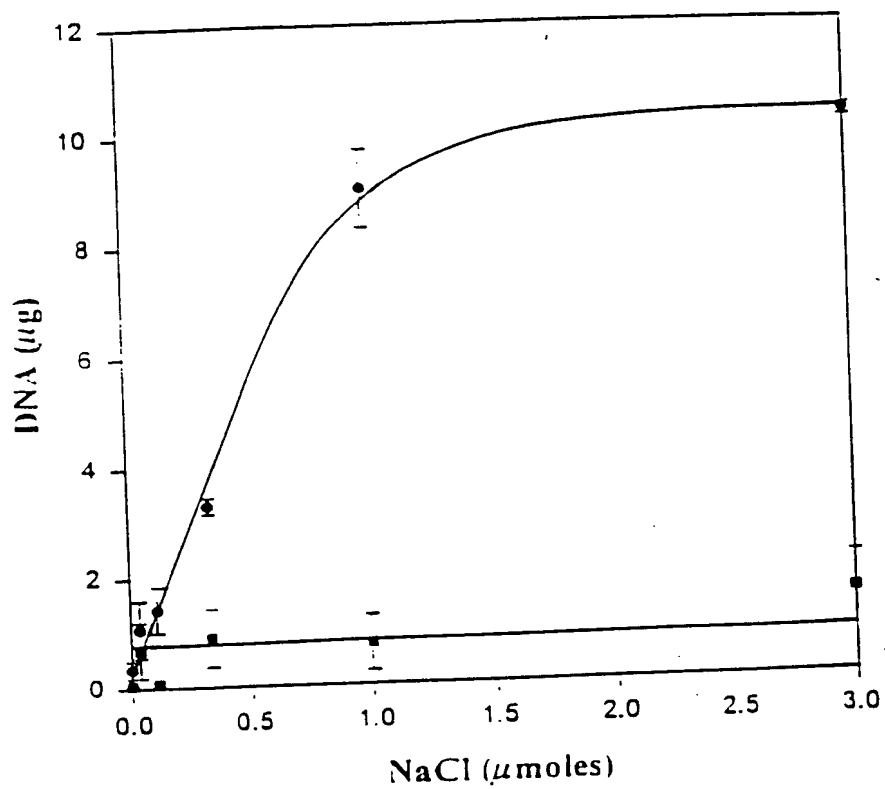
Figure 26A

Figure 26B



Effects of increasing amounts of OGP on the recovery of plasmid DNA from the aqueous (●) and solvent (■) phases following Bligh and Dyer extraction of

Figure 27



Effects of increasing amounts of NaCl on the recovery of plasmid DNA from the aqueous phase following Bligh and Dyer extraction of DNA/lipid complexes. Amount of DNA used was 10  $\mu$ g. DODAC (●), Lipofectamine (■).

Figure 28

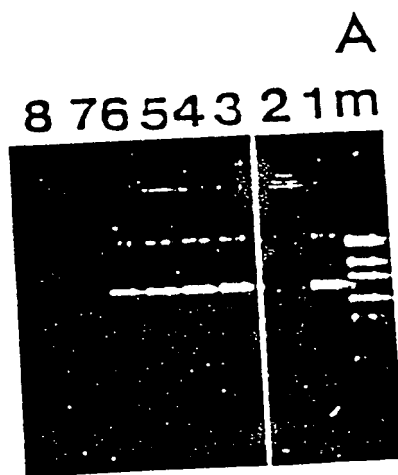


Figure 29A

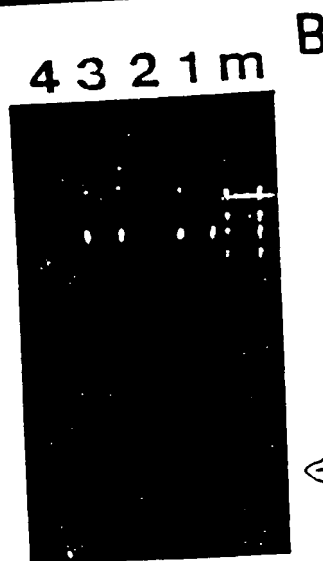
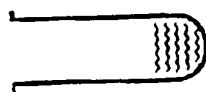


Figure 29B

551011-1651E450

10  $\mu$ g DNA  
in 200 $\mu$ l OGP



DNA/ESM  
in 600 $\mu$ l OGP

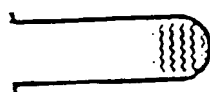


+

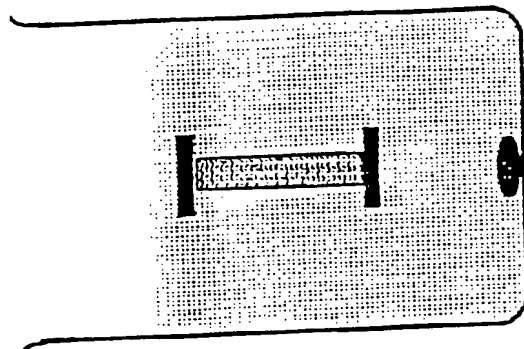
1/2HR



160nmol DODAC  
in 400 $\mu$ l OGP  
SONICATED



Dialyze in H<sub>2</sub>O  
for 3 days



160nmol ESM  
in 400 $\mu$ l OGP  
SONICATED

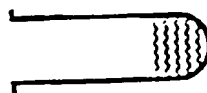


Figure 30

20 $\mu$ g  $\beta$ -gal DNA/160nmole DODAC/320nmole ESM

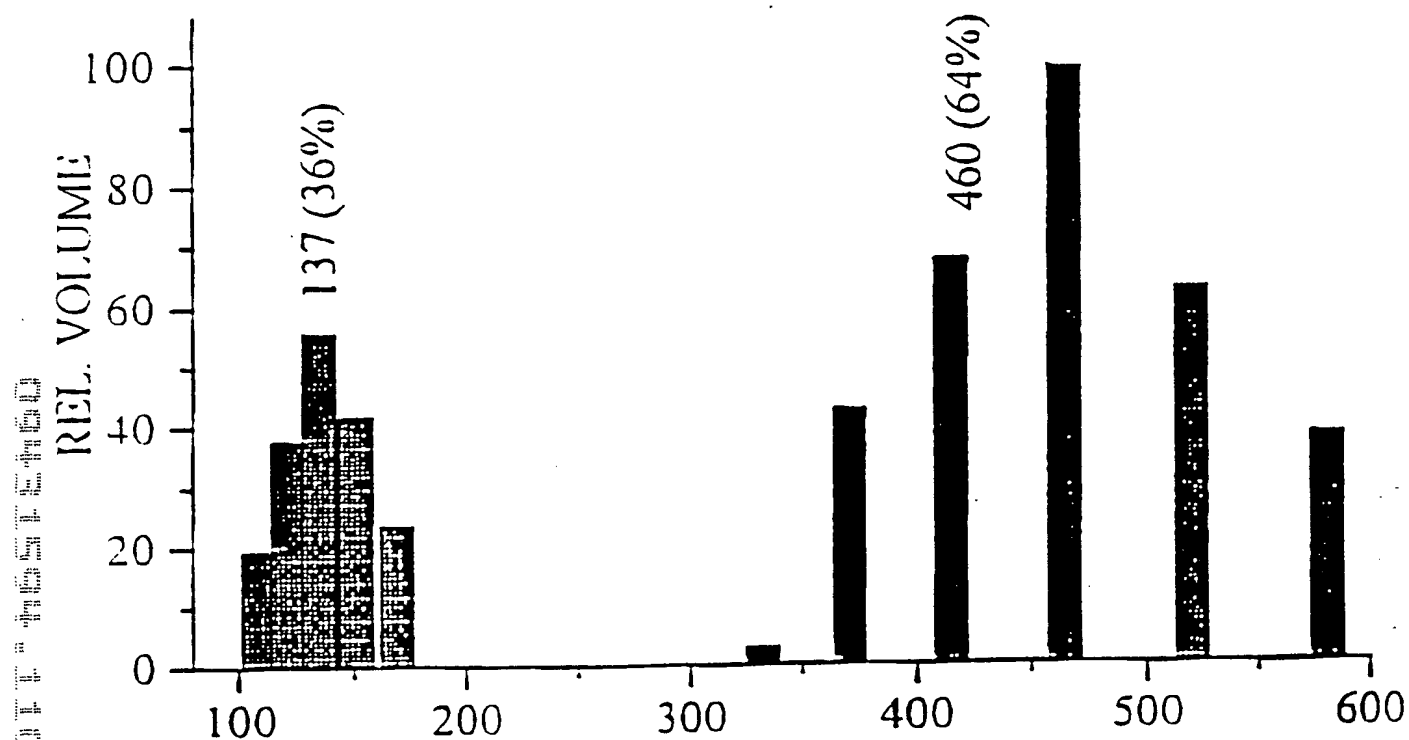


Figure 31A

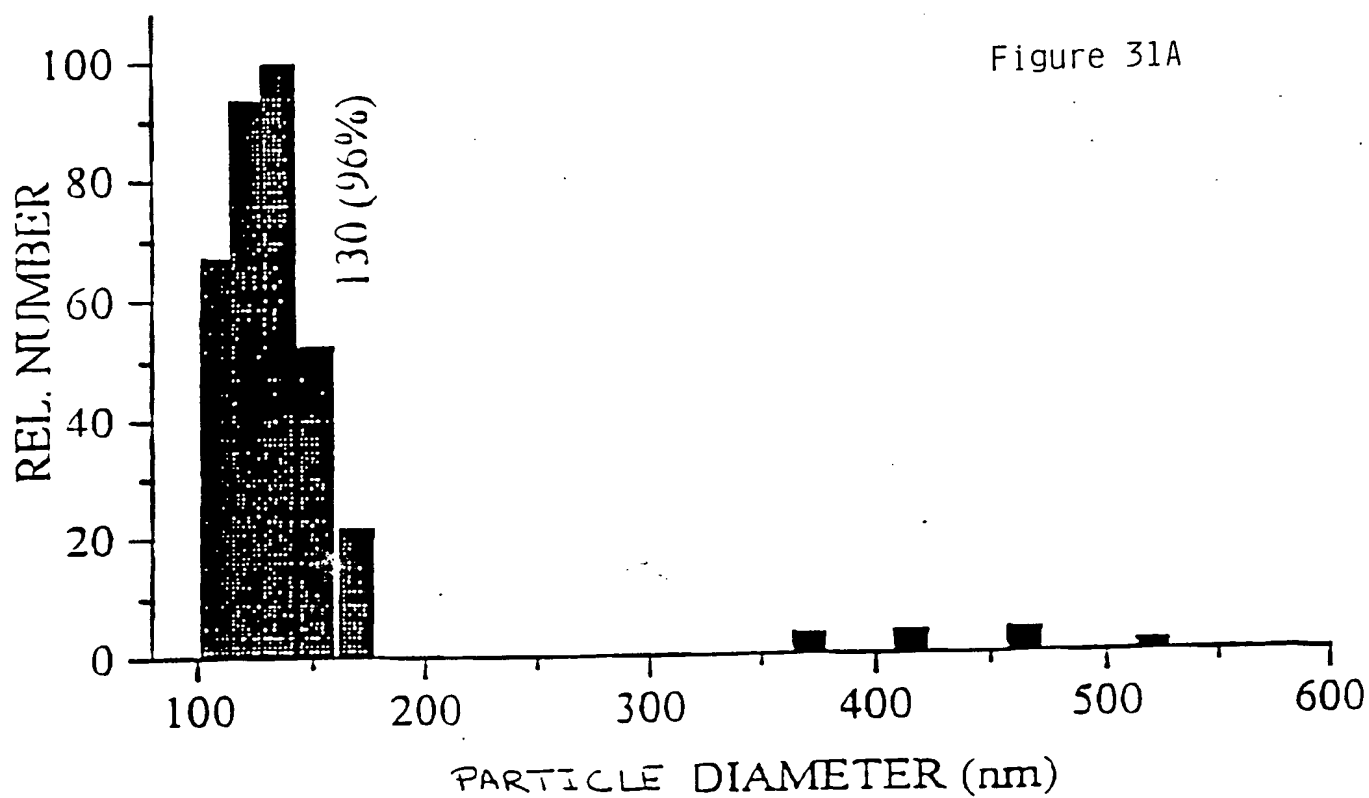


Figure 31B

--	--

DNA/TOPRO
-----------

DNA/OGP -TOPRO

1

DNA/DODAC.ESM+TOPRO-OGP 4:1

DNA/DODAC.DOPE--

DNA/DODAC.DOPE+TOPRO+OGP 4:1

111

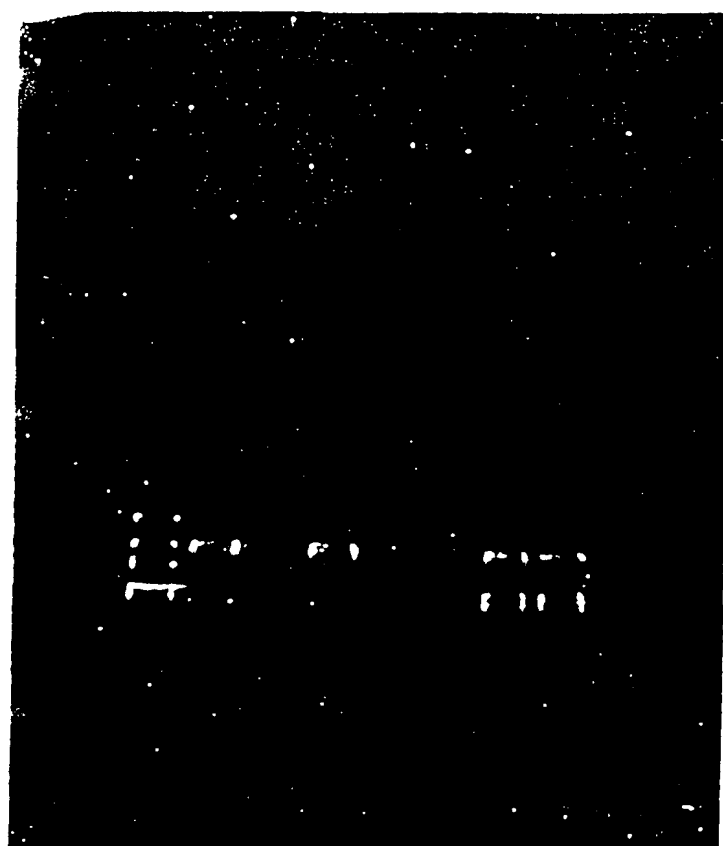
DNA/DODAC.DOPE-TOPRO+OGP 8:1

FLUORESCENCE INTENSITY

Figure 32



09431594-110159



M 3  
DNA  
+ DNase  
complex  
+ DNase  
LAP + DNase  
LAP + DNase

Figure 33

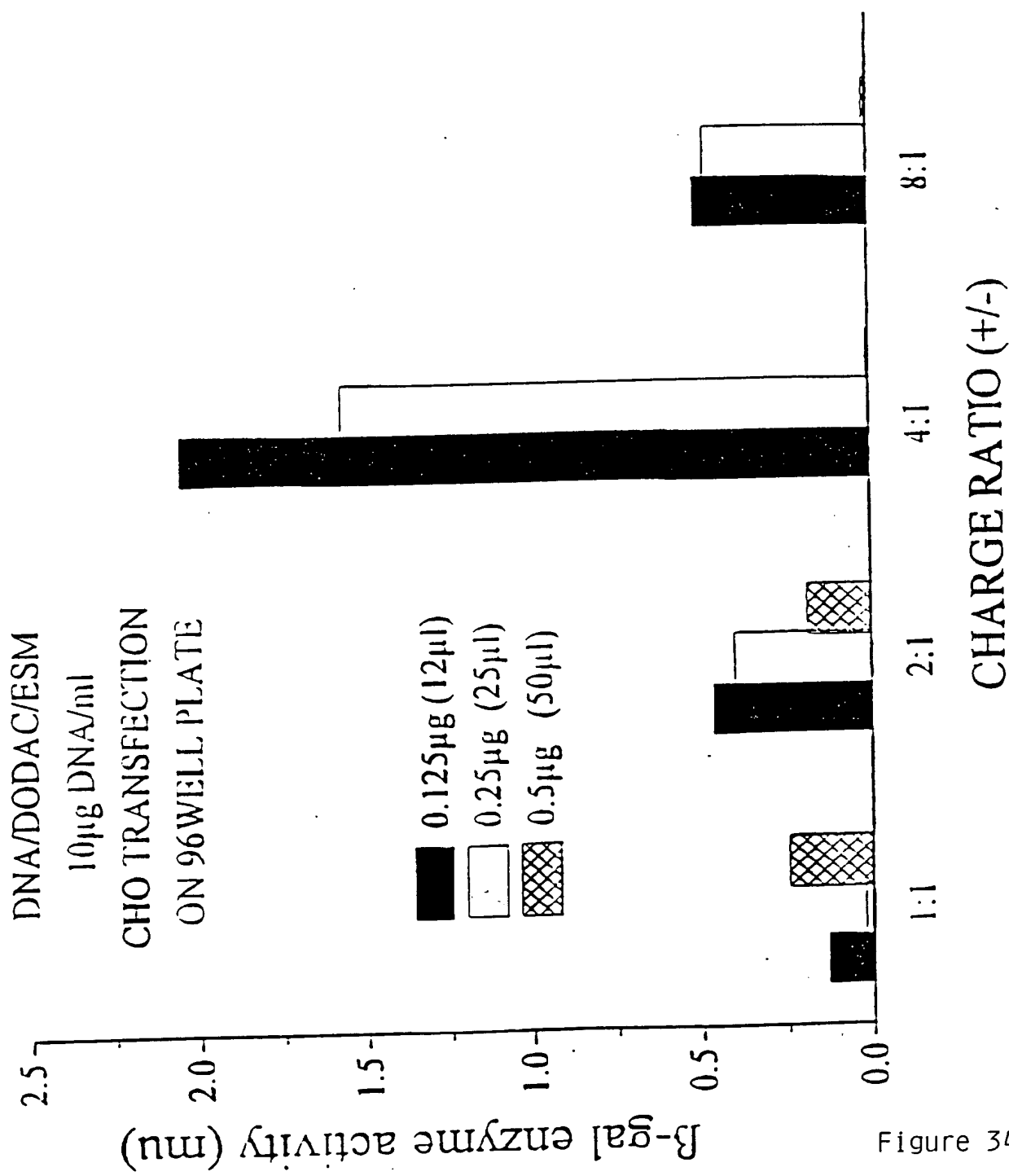


Figure 34

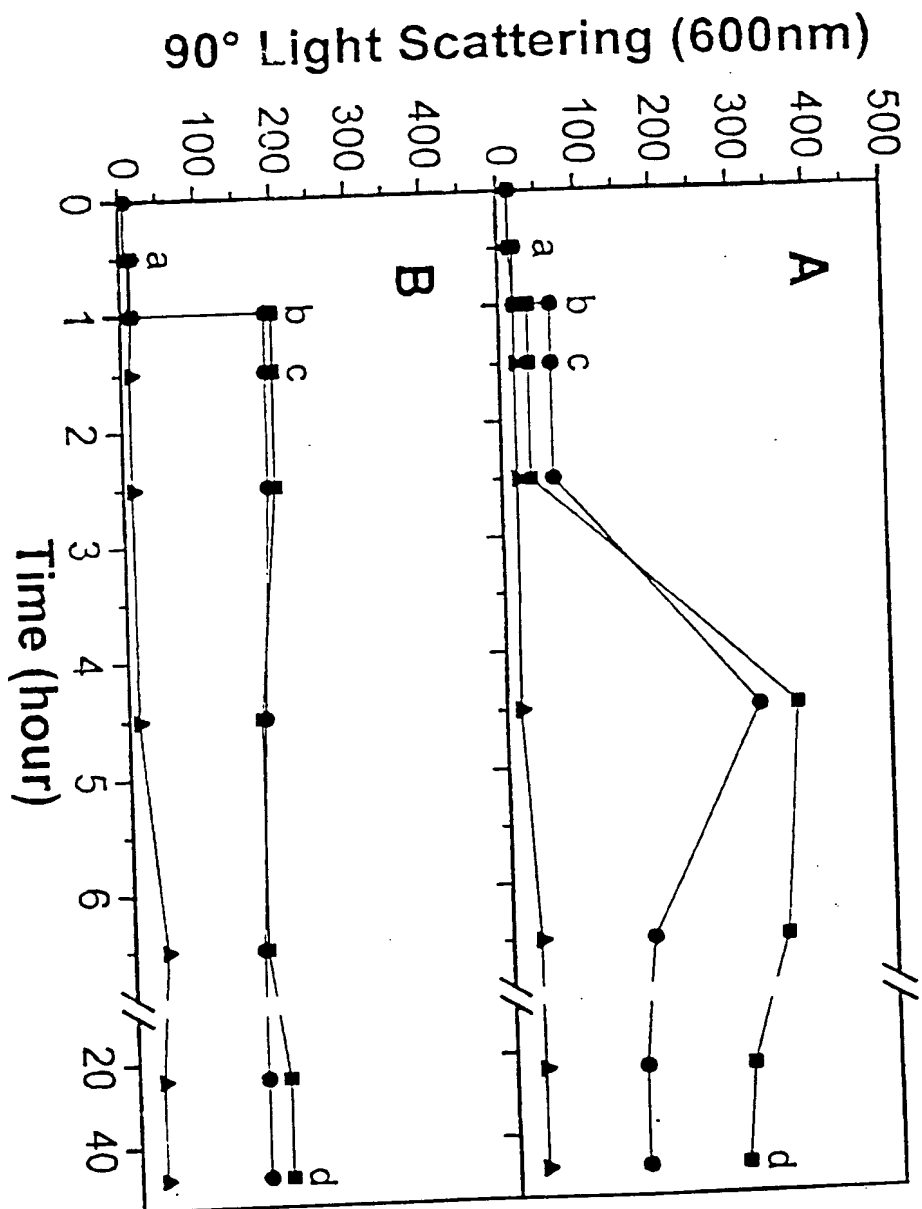


Figure 35A

Figure 35B

09431594-110199

# 90° Light Scattering (600nm)

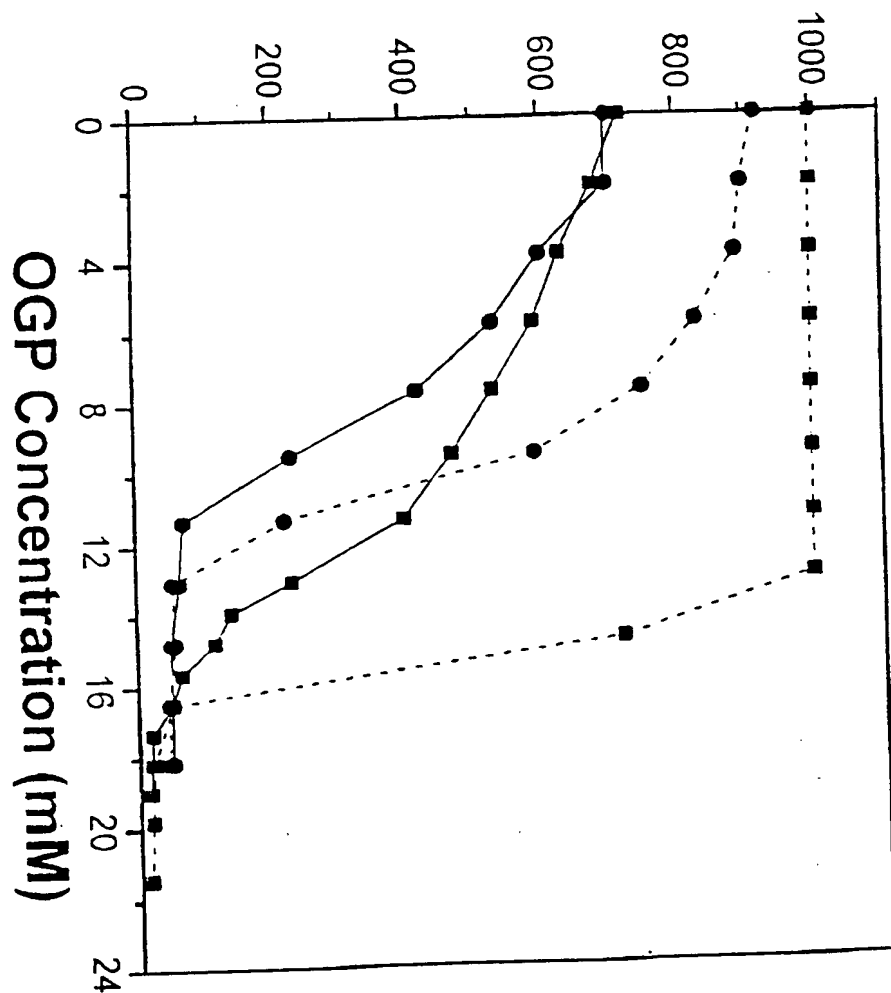


Figure 36

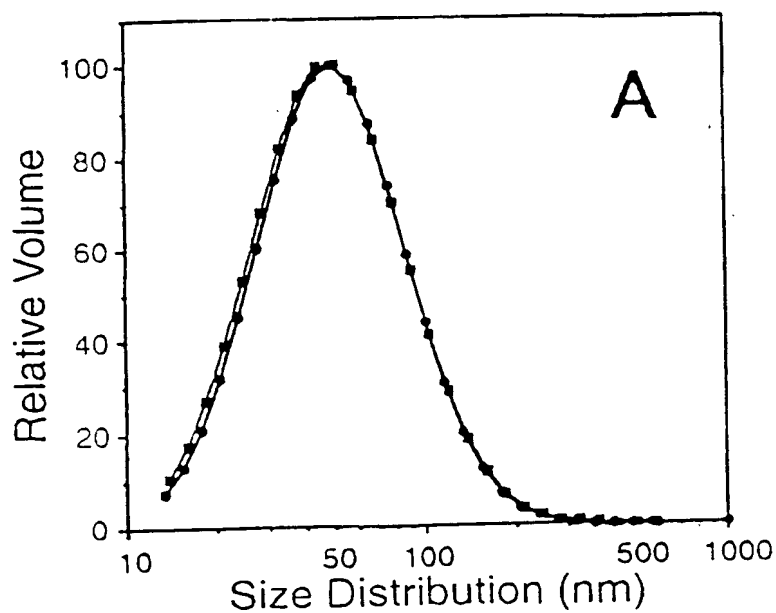


Figure 37A

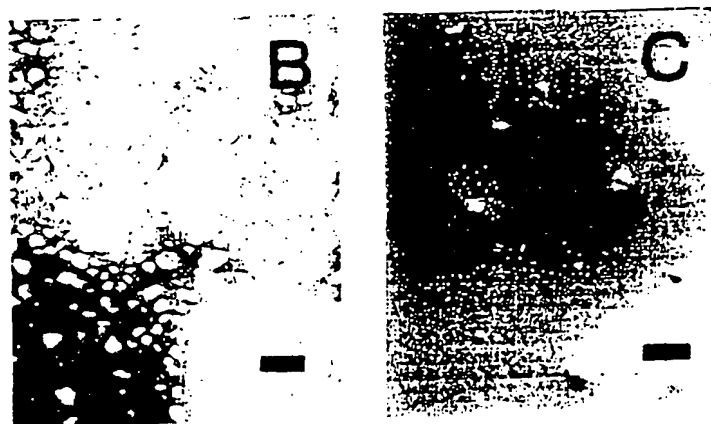


Figure 37B

Figure 37C

Figure 38B

B  
1 2 3 4 5

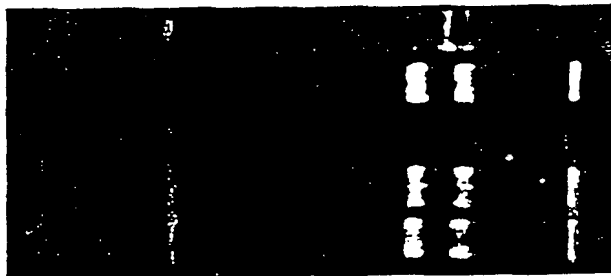
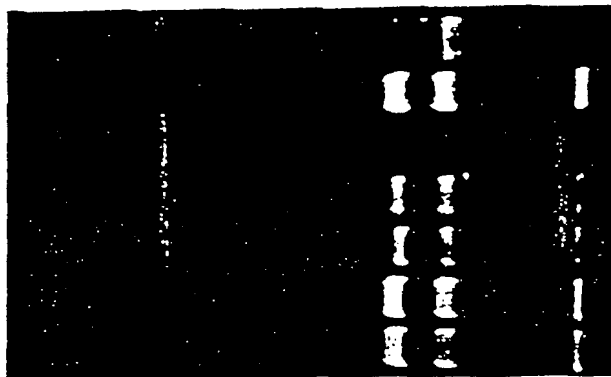


Figure 38A

A  
1 2 3 4 5 6 7



00431594-110199

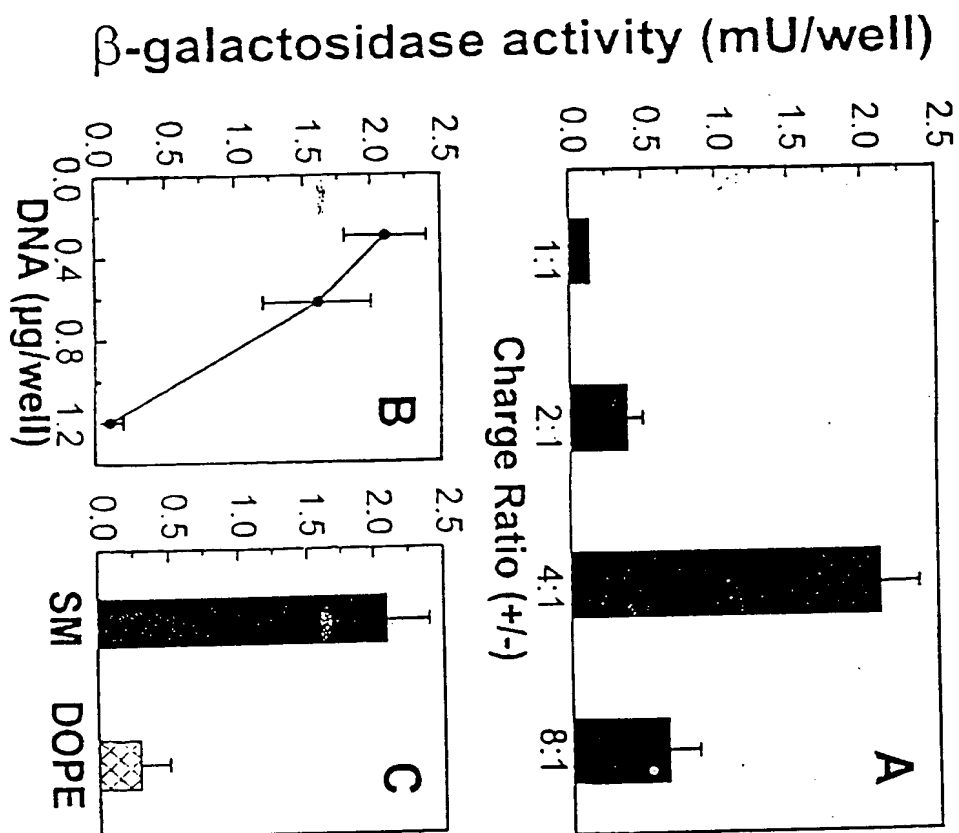
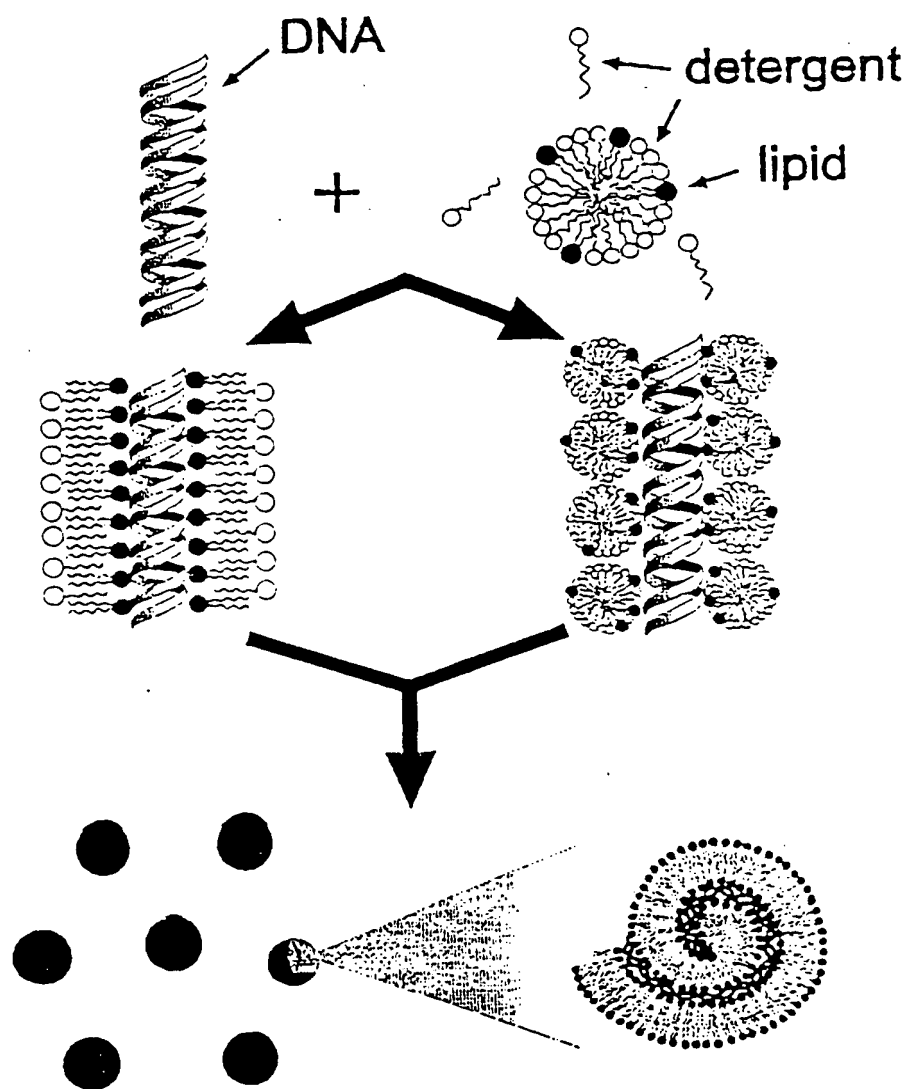


Figure 39A

Figure 39B

Figure 39C



DNA-Lipid Particle Formation

Figure 40



% Recovery of pINEXCAT with different composition of cationic lipid/DOPE/10 mol % PEG-CER C14 (5.0  $\mu$ mol total lipid) from DEAE Sepharose CL6B column in 150 mM NaCl, 20 mM HEPES (pH 7.4)

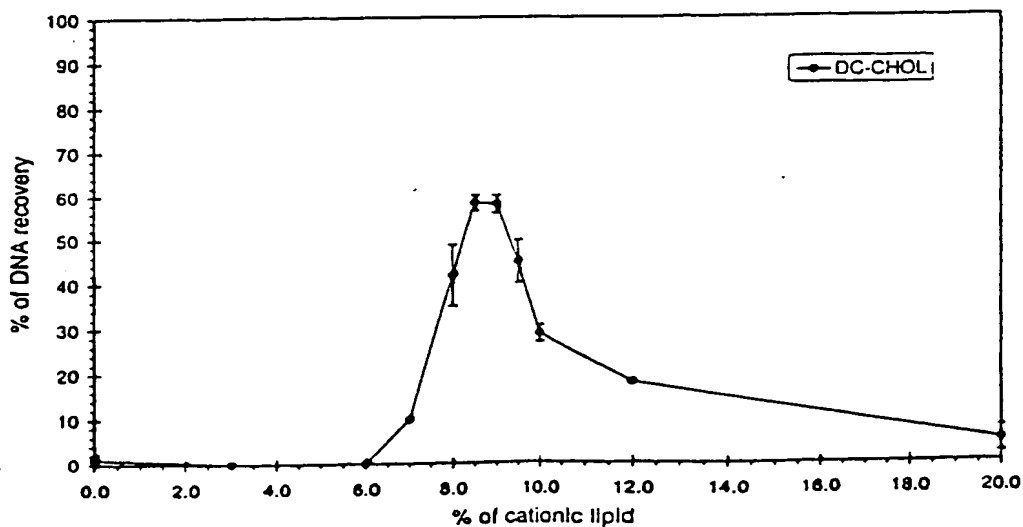
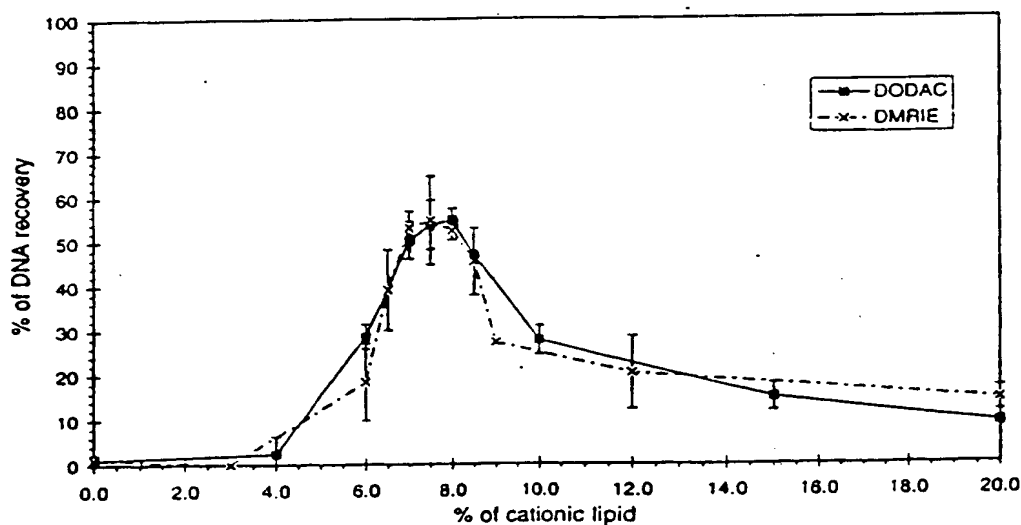
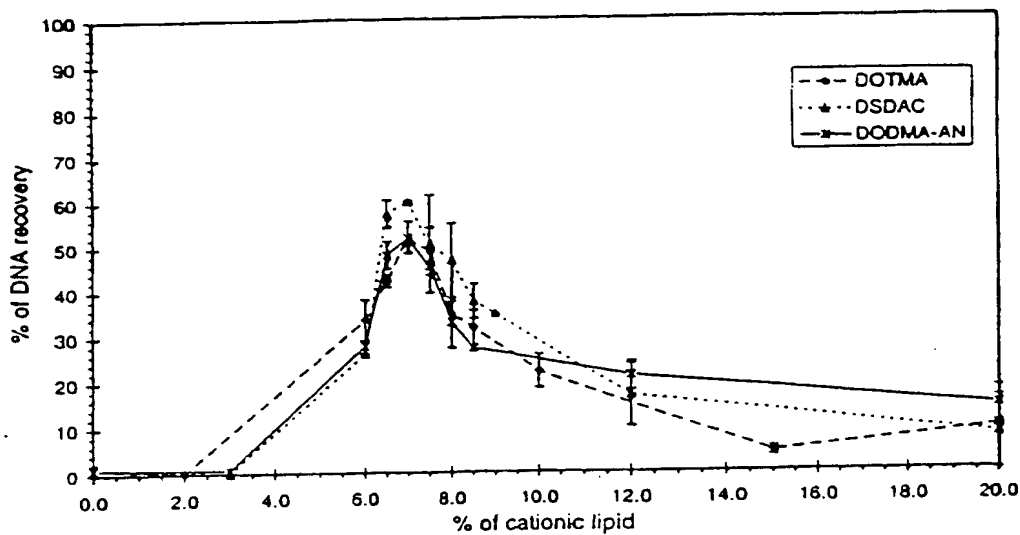


Figure 41A

Figure 41B

Figure 41C

Elution profile of 7.0 mol % DODAC/ 83 mol % DOPE/ 10 mol % PEG-CER C14  
from Sepharose CL4B column in 150 mM NaCl, 20 mM HEPES (pH 7.4)

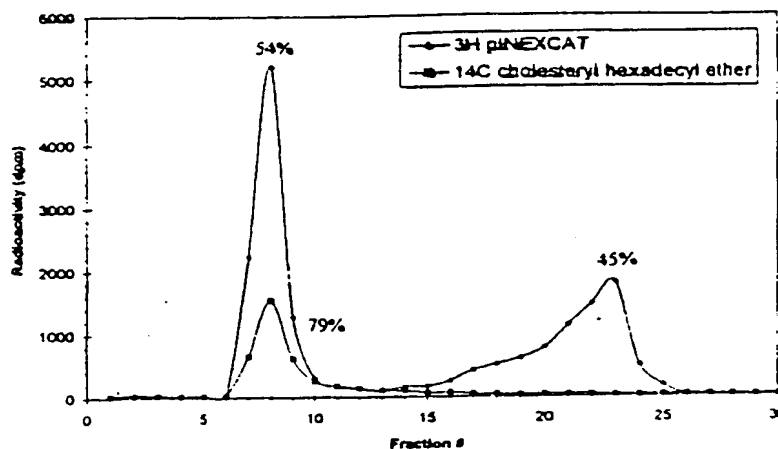


Figure 42A

(a)

Elution profile of 7.0 mol % DOTMA/ 83 mol % DOPE/ 10 mol % PEG-CER C14  
from Sepharose CL4B column in 150 mM NaCl, 20 mM HEPES (pH 7.4)

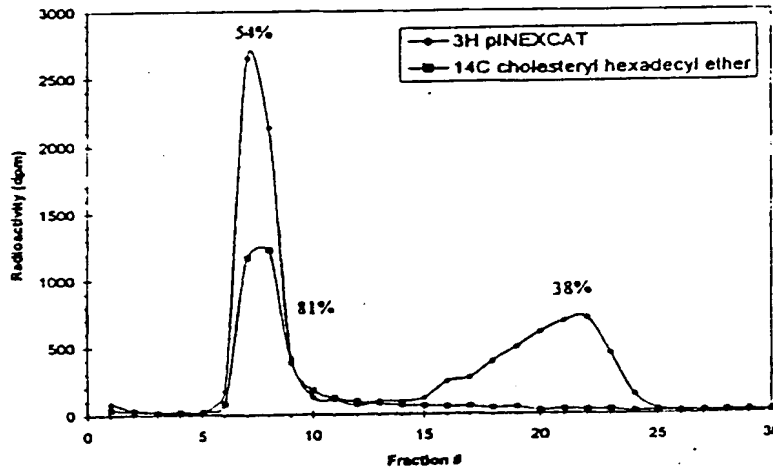


Figure 42B

(b)

Elution profile of 7.5 mol % DSDAC/ 82.5 mol % DOPE/ 10 mol % PEG-CER C14  
from Sepharose CL4B column in 150 mM NaCl, 20 mM HEPES (pH 7.4)

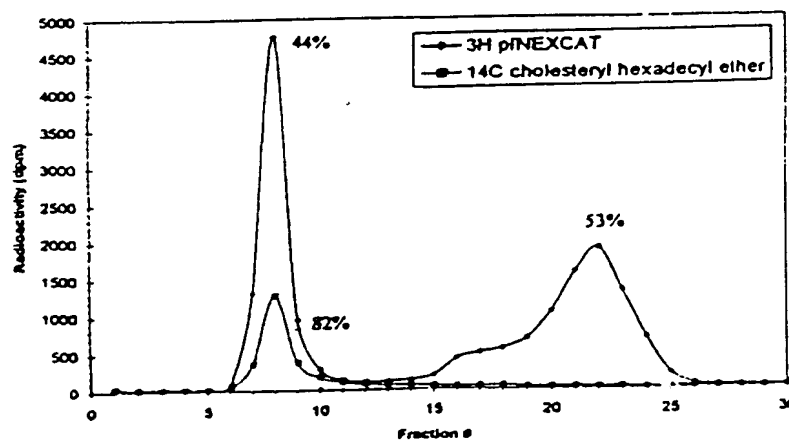


Figure 42C

(c)

EXAMPLE C

**Cationic Lipid Titration of 50 $\mu$ g pCMV $\beta$  in POPC:DOPE:PEG-CerC8:AL-1  
(65-x:25:10:x) Liposomes As Analyzed by the PicoGreen Assay**

Encapsulation Performed at pH4.8 or pH7.5

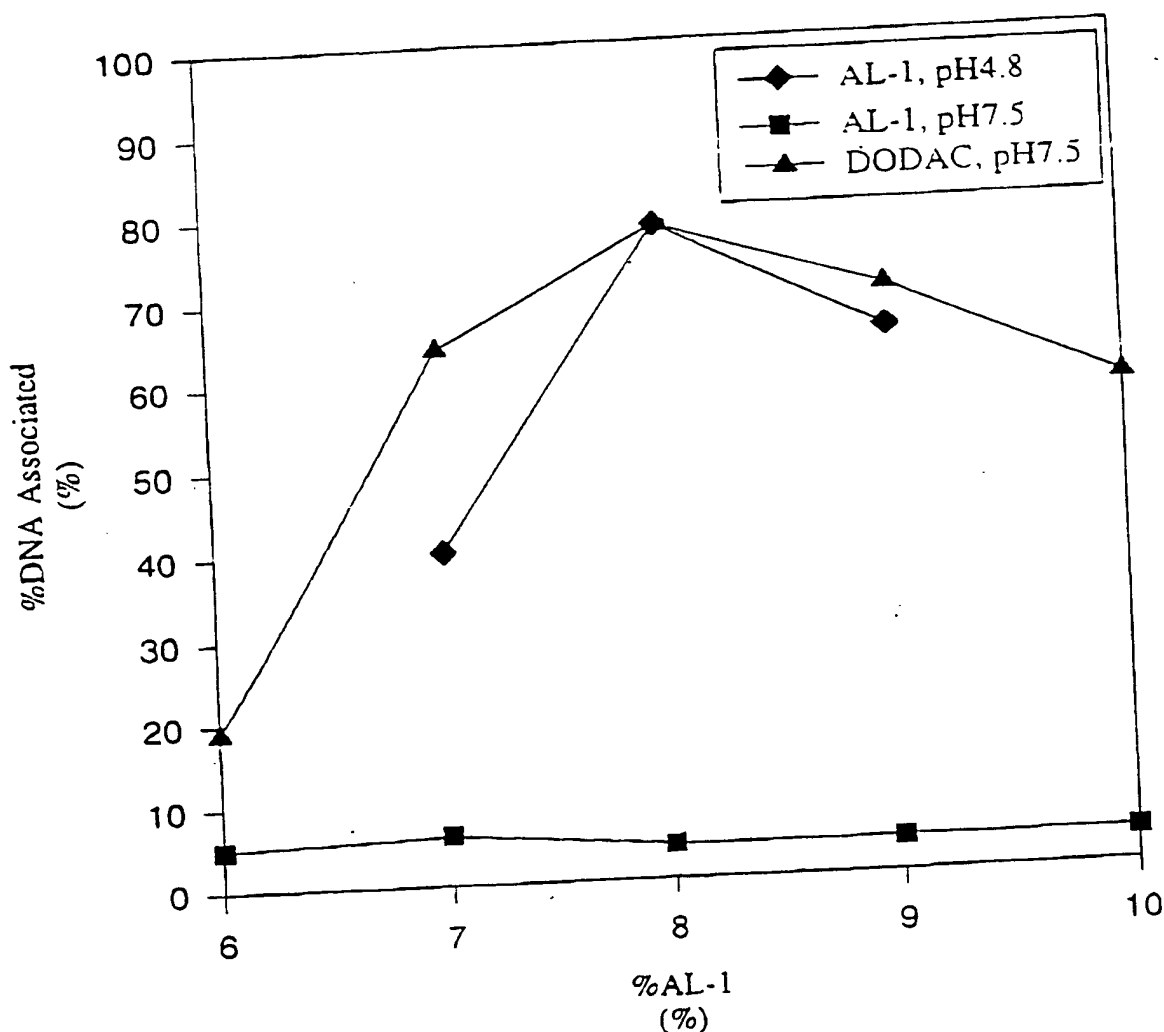


Figure 43

06/05/96 13:40 604 264 9959  
06/04/96 TUE 16:08 FAX 604 822 4343

INEX  
LIPOSM RES UNIT

007/009  
003

EXAMPLE D

(a)

Serum Stability (1.5 hr at 37°C) of 50µg pCMVβ  
Encapsulated in POPC:DOPE:PEG-CerC8:AL-1 (57:25:10:8)  
Liposomes {pH4.8 Encapsulation}

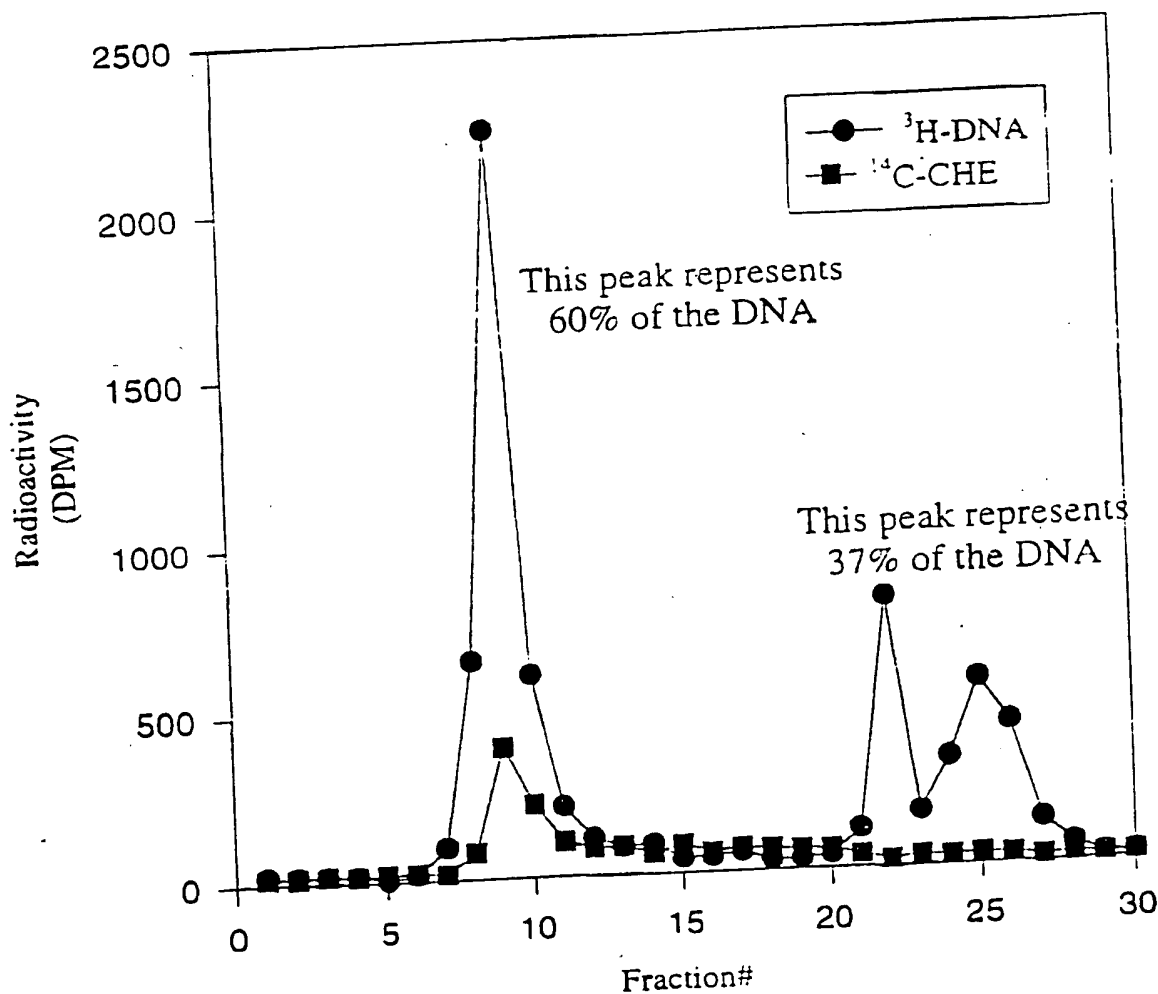


Figure 44

06/05/96 13:41 ☎604 264 9959  
06/04/96 TUE 16:03 FAX 604 922 4843

INEX  
LIPOSM RES UNIT

008/009  
004

EXAMPLE D  
(b)

**Serum Stability (1.5 hr at 37°C) of 50µg pCMVβ  
Encapsulated in POPC:DOPE:PEG-CerC8:AL-1 (57:25:10:8)  
Liposomes {pH7.5 Encapsulation}**

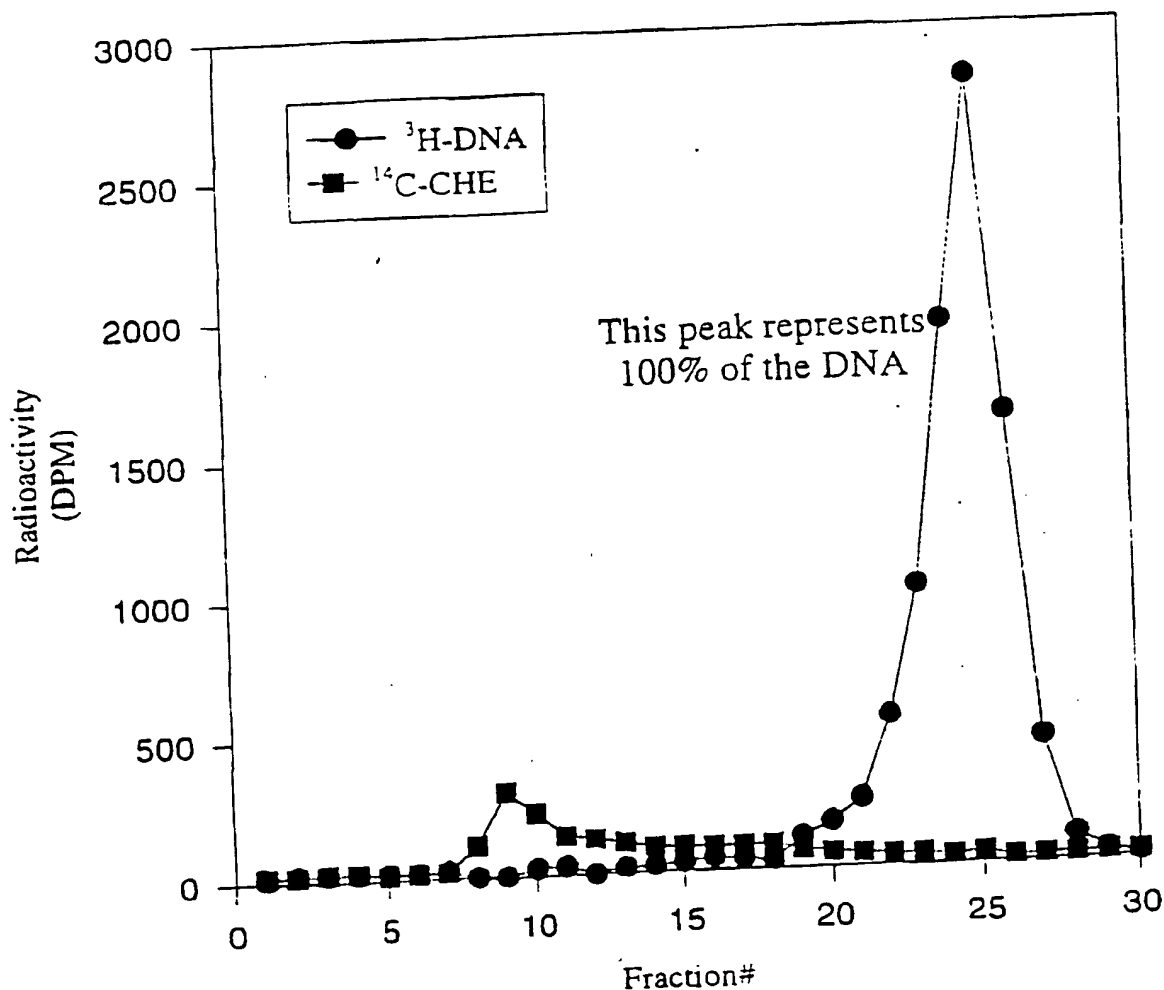


Figure 45

## EXAMPLE E

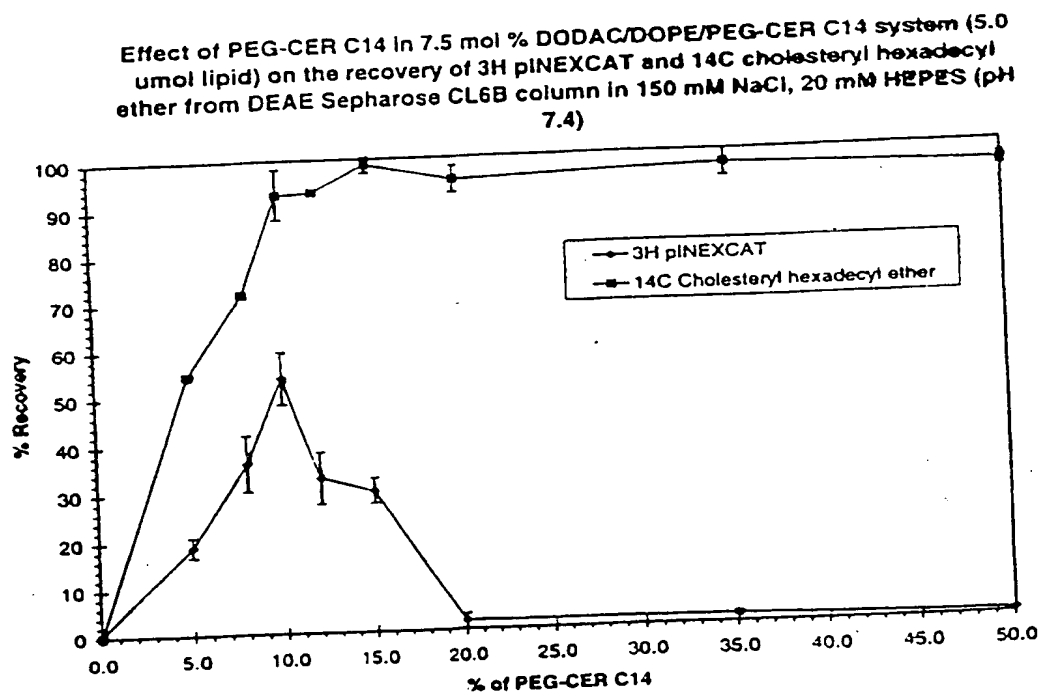


Figure 46